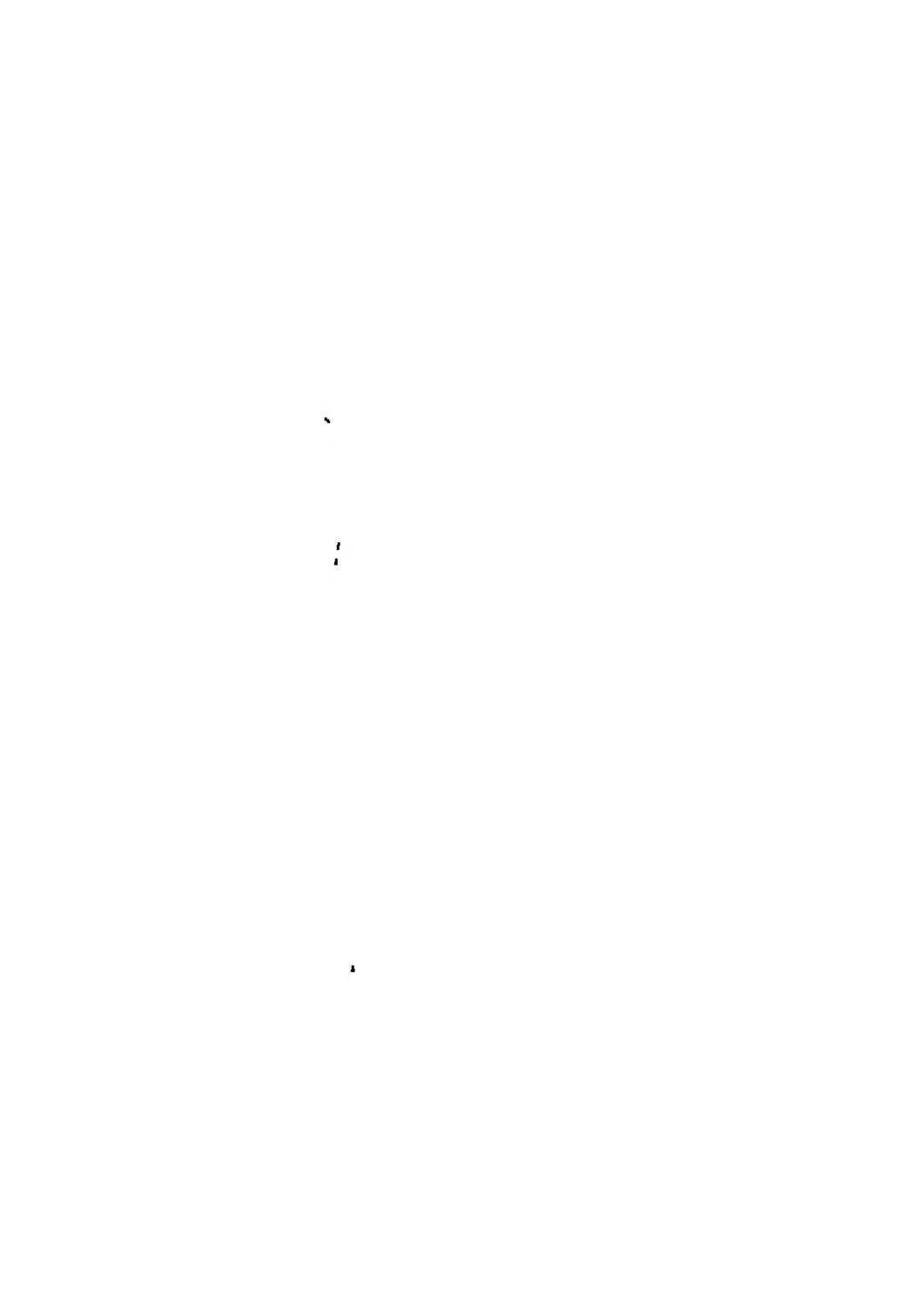




IMPERIAL INSTITUTE
OF
AGRICULTURAL RESEARCH, PUSA.

2018/136





CHEMICAL REVIEWS

GERALD WENDT, EDITOR

New York City

VOLUME VIII

2018



IARI

BALTIMORE
1931

CONTENTS

NUMBER 1, FEBRUARY, 1931

The Halogenation of the Paraffin Hydrocarbons. GUSTAV EGLOFF, R. E. SCHAAD AND C. D. LOWRY, JR.....	1
The Crystallization, Denaturation and Flocculation of Proteins with Special Reference to Albumin and Hemoglobin; together with an Appendix on the Physicochemical Behavior of Gly- cine. W. C. M. LEWIS.....	81

NUMBER 2, APRIL, 1931

SYMPOSIUM ON NON-AQUEOUS SOLUTIONS

Introduction to the Symposium. W. V. EVANS.....	167
An Introduction to the Chemistry of Acetic Acid Solutions. ARTHUR W. DAVIDSON.....	175
Acid-base Equilibria in Non-aqueous Solvents with Particular Reference to Glacial Acetic Acid. NORRIS F. HALL.....	191
Hydrogen Fluoride and its Solutions. J. H. SIMONS.....	213
Liquid Hydrogen Sulfide as a Reaction Medium. JOHN A. WILKINSON	237
Non-aqueous Reduction Reactions. CHARLES A. KRAUS.....	251
A Study of the Reducing Action of Sodium upon Salts in Liquid Ammonia Solution. WAYLAND M. BURGESS AND EDWARD H. SMOKER.....	265
The Properties of Solutions of Metals in Liquid Ammonia. WAR- REN C. JOHNSON AND ALBERT W. MEYER.....	273
The Behavior of Electrolytes in Dilute Methyl Alcohol Solution. JOHN WARREN WILLIAMS.....	303
Equilibria in Non-electrolyte Solutions in Relation to the Vapor Pressures and Densities of the Components. GEORGE SCATCHARD.....	321
Electrodeposition of Metals from Non-aqueous Solvents. L. F. AUDRIETH AND H. W. NELSON.....	335

NUMBER 3, JUNE, 1931

Polymerization. WALLACE H. CAROTHERS.....	353
The Energetics of Heterotrophic Bacteria. P. W. WILSON AND W. H. PETERSON.....	427

THE HALOGENATION OF THE PARAFFIN HYDRO-CARBONS¹

GUSTAV EGLOFF, RAYMOND E. SCHAAD AND C. D. LOWRY, JR.

Universal Oil Products Company Research Laboratories, Chicago, Illinois

Received April 15, 1930

CONTENTS

I. Introduction.....	3
II. Fluorination.....	5
III. Chlorination.....	6
1. Methane and natural gas.....	6
Photochemical.....	7
(a) Methane.....	7
(b) Patents.....	9
(c) Natural gas.....	11
Thermal.....	13
Catalytic.....	15
(a) Metallic salts and metals.....	15
(1) Methane.....	15
(2) Patents.....	18
(3) Natural gas.....	19
(b) Carbonaceous materials.....	21
(1) Methane.....	21
(2) Natural gas.....	21
(c) With chemical chlorinating agents.....	23
Electrical.....	24
(a) Methane	24
(b) Natural gas.....	24
2. Ethane.....	25
Photochemical.....	25
Thermal.....	27
Catalytic.....	27
Electrical.....	27
3. Propane.....	27
4. Butanes.....	29
n-Butane.....	29
Isobutane.....	30

¹ Presented before the Division of Organic Chemistry at the meeting of the American Chemical Society held at Columbus, Ohio, April 29 to May 3, 1929.

G. EGLOFF, R. E. SCHAAD AND C. D. LOWRY, JR.

5. Pentanes.....	32
<i>n</i> -Pentane.....	32
Isopentane.....	33
Tetramethylmethane.....	34
Pentane mixtures (petroleum fractions).....	35
(a) Photochemical chlorination.....	35
(b) Photochemical-catalytic chlorination.....	36
(c) Thermal chlorination.....	38
6. Hexanes.....	39
<i>n</i> -Hexane.....	39
2,3-Dimethylbutane (diisopropyl).....	42
7. Heptanes.....	43
<i>n</i> -Heptane.....	43
2-Methylhexane (isoheptane).....	45
8. Octanes.....	46
<i>n</i> -Octane.....	46
Other octanes.....	47
9. Nonane.....	48
10. Decanes.....	49
<i>n</i> -Decane.....	49
2,7-Dimethyloctane and decanes of unknown structure.....	51
11. Undecane.....	53
12. Dodecane.....	54
13. Tridecane.....	55
14. Tetradecane.....	56
15. Pentadecane.....	56
16. Hexadecane.....	57
17. Heptadecane.....	57
18. Octadecane.....	57
IV. Bromination.....	58
1. Methane.....	59
2. Ethane.....	60
3. Propane.....	60
4. Butanes.....	61
<i>n</i> -Butane.....	61
Isobutane.....	61
5. Pentanes.....	61
6. Hexanes.....	62
<i>n</i> -Hexane.....	62
2,3-Dimethylbutane (diisopropyl).....	64
7. Heptanes.....	64
<i>n</i> -Heptane.....	64
Isoheptane.....	66
8. Octane.....	67
9. Decanes.....	67
<i>n</i> -Decane.....	67
2,7-Dimethyloctane (diisoamyl).....	67
10. Dodecane	67

11. Tridecane.....	68
12. Pentadecane.....	68
V. Iodination.....	68
VI. Summary.....	68
VII. Suggested problems for further research.....	73
References.....	75

I. INTRODUCTION

The halogens, with the exception of iodine, are among the few chemical substances which react readily with the paraffin hydrocarbons. Fluorine reacts with avidity, chlorine very vigorously, and bromine, while less energetic in action, usually substitutes with no great difficulty. Iodine, as a rule, does not react with the paraffins, but one instance of iodination has been reported.

Direct fluorination has been studied only in the case of methane. Chlorination makes up the most voluminous, as well as the most important, chapter in the halogenation of the paraffins. The action of chlorine on all the normal hydrocarbons from methane to octadecane and a number of the compounds isomeric with these has been studied. Some of this work is of questionable value, because the supposedly pure hydrocarbons used were petroleum fractions. No matter how carefully prepared, there is reason to doubt the purity of such fractions, especially those of higher molecular weights, unless there is proof that they are chemical individuals.

Although failure to react with bromine is used as a diagnostic property of the paraffin hydrocarbons, it is found that on vigorous treatment, as with the aid of light, heat, or such catalysts as iron, charcoal, or iodine, substitution by bromine generally takes place. It has been claimed that the reactivity of the hydrocarbons towards this halogen decreases with the increase in the length of the carbon chain. No certain evidence of this has been offered, although the hydrocarbons above decane that have been studied have been reported to show little or no reaction with bromine.

Due to the availability of the lower paraffins, which abound in natural gas and petroleum, and the cheapness of chlorine, a considerable amount of study has been given to the industrial ap-

plication of chlorination. To some extent the chlorine derivatives of methane, particularly methyl chloride, are now manufactured from the hydrocarbon. The chlorination of pentane has developed a new source of amyl alcohol, amyl acetate, and isoprene.

In general, halogenation has been brought about by direct contact of the substituting element with the hydrocarbon, the latter in either gas or liquid form. Actinic light, heat, and catalysts have been extensively used to accelerate the reaction. Only a small amount of study has been given to the action on hydrocarbons of reagents containing loosely bound halogen, such as the chlorides and bromides of non-metallic elements.

Considerable difficulties are encountered in halogenating hydrocarbons, either for scientific purposes or in industrial practice. Chief among these are: the simultaneous formation of isomeric halides, rather than a single substance; the production of undesired polysubstitution products where monosubstitution is desired; and with chlorine, the danger of explosive reaction. Proper control of concentrations, temperature, and catalytic influences minimizes, though it does not entirely overcome, the first two difficulties; chlorination in the liquid phase, dilution of the hydrocarbon with carbon dioxide, nitrogen, steam, hydrogen chloride, or already chlorinated substances, and accurate regulation of the rate of addition of halogen greatly reduce the risk of explosion.

Herzfelder (62) has made certain generalizations in regard to chlorination and bromination:

"When into a monohalogen compound a second halogen atom is introduced, it always attaches itself to that carbon atom which is situated next to the carbon atom already united with halogen. In the case of further substitutions this rule only holds for bromine, of which it is never possible, by other than violent means, to attach more than one atom to each atom of carbon. On the other hand, when a third atom of chlorine is introduced, it frequently attaches itself to a carbon atom that is already united with chlorine.

"Bromides that already contain one atom of bromine united with each atom of carbon can not be easily further brominated; chlorides, however, take up more chlorine. A normal hydrocarbon, when brom-

inated by ordinary means, takes up as many atoms of bromine as it contains atoms of carbon; this was shown in the case of methane, ethane, propane, and also, although not with absolute certainty, in that of butane, hexane, and octane."

A study of the available experimental data on halogenation, however, leads one to the opinion that there is not sufficient evidence to justify such a sweeping statement.

In this review hydrocarbon fractions have been presented as individual compounds with the names given them by the original investigators. Boiling ranges and densities have been cited wherever possible and comparison of these with the constants of the pure hydrocarbons allows one to estimate to some extent the validity of conclusions drawn from the experimental work.

In many cases with both the hydrocarbon starting materials and the chlorinated products, the published identifications are not accurate. Recent work in hydrocarbon chemistry has shown how treacherous are hydrocarbon fractions, particularly from petroleum, when one is endeavoring to obtain pure substances, and how far they may be from single compounds even though boiling over narrow ranges and giving analytical values in harmony with supposed molecular composition.

II. FLUORINATION

The data available on the reaction of the paraffin hydrocarbons with fluorine are confined to two studies on methane. By passing a current of fluorine through a platinum tube into an atmosphere of this hydrocarbon Moissan (116) obtained a mixture of fluorine compounds from which he isolated carbon tetrafluoride. The reaction was accompanied by incandescence and deposition of carbon.

Later Moissan and Chavanne (117) observed that on coming in contact with liquid fluorine at -187°C . solid methane reacted with a bright flash and violent explosion. It was impossible to identify the products because the glass reaction tubes were "pulverized."

III. CHLORINATION

1. *Methane and natural gas*

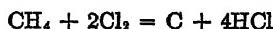
Chlorination of methane results in the formation of all the possible products—methyl chloride, dichloromethane, chloroform, and carbon tetrachloride. Bedford (14) reported that at low temperatures in the presence of actinic light each of these compounds forms in turn from the preceding member of the series, chlorine showing a slight preference for chloromethanes over the hydrocarbon itself. As a result, the product of reaction is usually not a single substance, but a mixture of several compounds. The relative proportions of the derivatives obtained are determined largely by the ratio of chlorine to hydrocarbon in the mixture of reacting gases. Other conditions, such as small changes in temperature or the presence of catalysts, have influence in lesser degree.

Martin and Fuchs (108) endeavored to calculate from thermochemical data the ratios which may be expected between the chloromethanes after the reaction of chlorine and methane. They believed that under the same conditions the ratio of the velocity constants of the successive stages of chlorination should be proportional to the number of hydrogen atoms displaced by chlorine. On the basis of these theoretical ratios (4:3:2:1), the course of the chlorination of methane with time was calculated. The theoretical and experimental results were in agreement in the neighborhood of 300°; at higher temperatures, especially in the presence of catalysts which presented large surfaces, the more highly chlorinated products were formed in greater proportions than was demanded by theory. The work of other investigators has not borne out the hypothesis of Martin and Fuchs.

Müller (120) has suggested that in chlorination the methane and chlorine may first combine to form an addition compound, which then splits to a chlorine derivative and hydrochloric acid. During chlorination there is also a condensing action, resulting in the formation from methane of halogen derivatives of higher hydrocarbons, especially of ethane.

If the chlorination is not controlled, but allowed to cause a

considerable elevation of temperature by reason of its exothermic nature, the course of the reaction changes. Substitution gives way to a disruptive process, producing free carbon and hydrogen chloride according to the equation:



The chlorination of methane has been accelerated in many different ways. Actinic light has been used frequently. Some investigators have employed catalysts, or used a chlorine hydrocarbon mixture raised to temperatures as high as 600°, or subjected the mixture to the silent electrical discharge. At times two or more of these methods of stimulating reaction have been allowed to act simultaneously.

Photochemical chlorination. (a) *Methane.* Sunlight, diffused daylight, light from a carbon arc, from a metallic filament and from a mercury-vapor lamp, have been used to promote the chlorination of methane. In one study on natural gas (12) an interesting comparison of the influence of light of different wave lengths on chlorination was made. Blue light had by far the greatest accelerating effect. Most other colors, even ultra-violet, were ineffective. Some workers have recommended "ultra-violet lamps" as a source of activating light in chlorination. It is quite probable, however, that light from these lamps of wave lengths other than those of ultra-violet is the effective agent assisting reaction with the halogen.

In most of the experimental work that has been reported the halogen and hydrocarbon were treated at room temperature. According to patent claims, however, where methyl chloride is sought the reaction chamber may be cooled, and in some work the chlorination has been carried out in the interstices between ice cakes.

Dumas (42) observed that while methane and chlorine could be mixed in all proportions in the dark without any immediate action, the mixing of three volumes of chlorine and one of methane in daylight, even when diffused, produced a violent explosion. If the methane, however, was first diluted with an equal volume of carbon dioxide, the chlorine could be added without danger,

and when methane was permitted to diffuse downward into chlorine in the presence of sunlight, the reaction proceeded smoothly. Carbon tetrachloride and a smaller amount of chloroform were produced under these conditions. Attempts to produce methyl chloride by the action of either gaseous chlorine, antimony pentachloride, or phosphorus pentachloride upon methane were unsuccessful.

Bischoff (18) found that a mixture of methane with twice its volume of chlorine exploded in bright sunlight, producing carbon and hydrogen chloride. If the light intensity was weakened by clouds, while the reaction occurred, carbon was not deposited and the residual gas had the odor of turpentine. Berthelot (16) also observed that the use of diffused sunlight was necessary in order to obtain normal chlorination, as direct sunlight nearly always caused explosion.

A little later, Kolbe and Varrentrap (82) noted that in diffused light equal volumes of dry methane and chlorine were converted into equal volumes of hydrogen chloride and a chlorine-containing, combustible gas. They were unable to decide whether this was "actually methyl chloride or an isomeric compound."

In contrast with previous observations, Phillips (129) reported that "a mixture of methane and chlorine was exposed over water to bright sunshine on a July day without undergoing any noticeable contraction in volume or change in color." He claimed that chlorine attacked methane only at temperatures considerably above 100°.

Whiston (192) mixed chlorine and methane in various proportions in the dark and exposed the mixtures to daylight admitted through glass windows. In a mixture of 2.75 volumes of methane to 1 volume of chlorine, he found that not more than 25 per cent of the chlorine reacting went to form methyl chloride, while from a mixture of equal volumes of the two gases only 10 to 13 per cent of the chlorine appeared in the monohalogenated product. Whiston did not determine the amounts of dichloromethane, chloroform, and carbon tetrachloride produced.

In preliminary experiments Whiston (192) found that in mixtures containing two volumes of methane to one of chlorine

there was no action in the dark, a slow combination under the influence of light from an ordinary 60-watt metallic filament lamp placed 30 cm. from the reaction vessel, and a much more rapid reaction when an arc light was used. If a very small amount of nitrosyl chloride was present, practically no action took place even in the strongest light.

Römer (138) found that methane when illuminated was chlorinated more readily than hydrogen in a mixture of the two gases. He found it impossible, however, to predict the partition of the chlorine between the gases from a study of the velocity of their individual reactions.

(b) *Patents on photochemical chlorination of methane.* The industrial interest taken in the photochemical chlorination of methane is shown by the number of patents which have been issued on this subject. Elworthy and Lance (46) claimed to be able to prepare chloroform by mixing "suitable proportions" of methane and chlorine, adding an inert diluent as nitrogen or carbon dioxide, and subjecting the mixture to the action of sunlight or other suitable illumination in a series of thick-walled glass tubes. The dilution was said to eliminate all danger of explosion.

By Walter's process (189) methane was mixed with a corresponding quantity of chlorine and exposed in air-cooled glass tubes to light of gradually increasing intensity. It was stated that the reaction could also be carried out satisfactorily in a tower-like reaction chamber in which the light source was overhead. Walter claimed that by working with a gas mixture consisting of one volume of methane to four of chlorine about 45 per cent of the weight of chlorine was used, and methyl chloride, dichloromethane, and chloroform as well as carbon tetrachloride were formed, the proportions varying with the quantity of chlorine and the reaction temperature.

Snelling (172) introduced mixtures of methane and chlorine, which would ordinarily be explosive, into dome-shaped glass vessels two feet in diameter and five feet high, and exposed them to intense light. He claimed that explosion was prevented by convection currents set up by the heat liberated in the reaction.

The reaction was started at a low rate of gas flow, and after it had commenced the mixture of methane and chlorine was admitted at rates up to 60 liters per minute with complete and quiet combination. The products were dichloromethane, chloroform, and carbon tetrachloride.

In a variation of this process, Snelling (170) passed mixtures of methane and chlorine through three parallel series of transparent, dome-shaped reaction vessels (171). As the gas advanced through the apparatus it was exposed to actinic light (as from mercury-vapor lamps) of gradually increasing intensity.

According to another patent (173) of Snelling, chlorine was mixed with an excess of methane, reaction brought about, and the resulting gases brought in contact with a solvent for removing the methyl chloride, such as oil or carbon tetrachloride, which is a relatively poor solvent for the unhalogenated hydrocarbon. The residual methane was then retreated, and the circulation continued.

Riesenfeld (136) developed an apparatus for chlorinating methane with provision for preventing sudden increase or stoppage of the flow of the gas-mixture into and out of the reaction chamber, which would be likely to cause explosion. He also claimed that he could regulate the chlorinated product by varying the speeds of flow of methane and chlorine. Reaction of equal volumes of the gases was said to give 80 per cent of methyl chloride, 10 per cent of dichloromethane, and small amounts of chloroform, carbon tetrachloride, and hexachloroethane. When the chlorine-methane ratio was between 3:1 and 5:1, the product consisted of 5 per cent of chloroform, 42 per cent of carbon tetrachloride, and 10 per cent of chloroethanes.

Leiser and Ziffer (99) aimed to obtain mainly methyl chloride by rapidly passing a mixture of one volume of chlorine to six of methane through four reaction chambers of progressively increasing size. During the passage, the reacting gases were exposed to light from mercury-vapor lamps extending axially through the chambers. Addition to the gas mixtures of small amounts of hydrogen chloride proved advantageous, while a small amount of water vapor seemed to prevent the undesired disruptive reaction to hydrogen chloride and free carbon.

The Goldschmidt A.-G. (56) patented a process for the production of methyl alcohol and dichloromethane, which consisted in chlorinating methane and treating the chlorinated product under pressure with milk of lime. Methyl alcohol was formed by action of the milk of lime on methyl chloride and was carried away dissolved in the lime suspension, while the dichloromethane was carried by the gas stream and later separated by cooling to low temperature.

(c) *Natural gas.* By employing Dumas's method (42) of allowing chlorine and the hydrocarbon gas to mix slowly by diffusion while exposed to sunlight, Melsens (109) prepared carbon tetrachloride from marsh gas and thus proved its identity with methane produced from the acetates.

Baskerville and Riederer (12) made the study already referred to concerning the wave length of light used to speed the reaction of natural gas and chlorine mixtures. The radiation from a "Uviol" ultra-violet lamp was found to have no noticeable effect on the chlorination. Light from either an unscreened carbon arc or from the arc screened with blue accelerated the reaction, but the interposition of green, yellow, or red screens decreased the rate of chlorination almost immediately. When the blue screen replaced these, the reaction was at once reaccelerated. The screening-out of the other colors was of no assistance to the chlorination, as the inactive red, yellow, and green rays had little, if any, interfering influence. In other experiments natural gas mixed with chlorine was heated and passed into a reaction chamber consisting of a cruciform glass tube, through two opposite arms of which a quartz tube containing iron terminals was inserted, across which an arc was struck. A yield of approximately 20 to 25 per cent of the methane as chloroform and carbon tetrachloride mixture was obtained.

Tolloczko (182) chlorinated natural gas in the outer jacket of an ultra-violet lamp constructed in the form of a Liebig condenser. The reaction proceeded continuously at 80–100° without explosion, even with equal volumes of natural gas and chlorine, but more slowly with the lamp than in sunlight (183). An excess of natural gas (natural gas: chlorine = 1.2:1) gave with double pass-

age of the gases between the walls of the ultra-violet lamp, a yield of 1.53 grams of oil and 0.38 grams of gaseous products per liter of natural gas. The volatile products consisted mainly of methyl chloride and dichloromethane. The oily portion was separated into dichloromethane and chloroform, which in nearly equal amounts made up 75 per cent of the oil, carbon tetrachloride, dichloroethane, ethylene dichloride, and tetrachloroethane. Chlorination with an excess of halogen yielded solid hexachloroethane as well as oily products.

Bedford (14) exposed a mixture of natural gas and chlorine, confined over water at room temperature, to light from a "white flame arc," and then cooled the gas by shaking with the water. He found that for the production of the lower chloro derivatives of methane, the chlorine should be added in small increments, and after each addition the reaction should be completed and the heat of reaction dissipated before more chlorine was used.

In order to obtain better cooling and to prevent the formation of carbon tetrachloride, Bedford later carried out the chlorination in the interstices between cakes of ice in a large Woulff bottle. The reaction was started by means of the white flame arc in a mixture containing about 12.5 per cent by volume of chlorine. The hydrocarbon gas and chlorine were then admitted in regulated amounts to the Woulff bottle while the reaction proceeded. Bedford obtained 166 cc. of a mixture of dichloromethane and chloroform from 2.6 cubic feet of natural gas. This was equivalent to about 17 gallons per 1000 cubic feet.

In a large wooden reaction chamber provided with a plate glass window through which the mixture was exposed to light from the white flame arc, 250 cubic feet of natural gas was consumed at a rate of 14 to 30 cubic feet per hour, and gave several gallons of product, which was separated under water. The product contained 35 per cent by volume of dichloromethane, 35 per cent of chloroform, 5 per cent of carbon tetrachloride, and 20 per cent of chloroethanes. About 14 per cent of the reaction product dissolved in the water resulting from the melting of the ice by the heat of the reaction. This portion of the product contained 61 per cent of dichloromethane, 28 per cent of chloroform, 1.5 per cent of carbon tetrachloride and 6 per cent of chloroethanes.

Bedford (15) patented the process of chlorination in the interstices between cakes of ice. He claimed that methyl chloride was the main product when the reaction chamber was full of cakes of ice. The use of finely cracked ice reduced the speed of chlorination and favored the formation of dichloromethane. Higher average temperature produced approximately half dichloromethane and half chloroform.

Patents of Snelling (167, 168) employed actinic light, the intensity of which increased as the gases moved through the apparatus. Natural gas containing ethane and propane as well as methane was passed, together with chlorine, toward sunlight or some artificial actinic light in reaction vessels containing a series of transparent baffles. In this way the mixture richest in chlorine was exposed to light of the least intensity. The temperature within the reaction cell was controlled and the reaction mixture diluted if desired by an inactive gas such as steam, carbon dioxide, nitrogen, or highly chlorinated (169) hydrocarbons.

Thermal chlorination. In some methane chlorinations, particularly processes covered in patents, reaction has been assisted by heat. The low limit of effectiveness of heat appears to be about 250°, and work has been reported up to as high as 500°. In the presence of light the reacting gases have also sometimes been heated, but to lower temperatures.

Jones, Allison and Meighan (76) reported the beginning of chlorination in the dark at 250°. Lacy (88) stated that a chlorinated product consisting mainly of methyl chloride was obtained by passage of an excess of methane mixed in the cold with chlorine (a ratio of ten volumes of methane to one of chlorine was suggested) through a reaction vessel of glass, quartz, porcelain, or earthenware maintained at a temperature of about 400°.

In further patents Lacy (89, 95) claimed that both methyl chloride and dichloromethane were formed by the use of from four to eight volumes of methane to one of chlorine. Moreover the heat developed by the reaction was sufficient to maintain the gas mixture at a temperature of 400–600°. Use of a porous contact material in the reaction chamber accelerated the heating of the incoming gases and increased the velocity of reaction.

Lacy (96, 97) also found that reaction could be effected by mixing chlorine at ordinary temperatures with an excess of methane, and then introducing an additional quantity of the hydrocarbon at an elevated temperature. An apparatus (92) was proposed which comprised an iron shell lined with "acid tile" brick or silica, surrounded by a flue, and heated by gas. A filling of ground flint was placed between the shell and the lining. Conditions and temperatures were regulated so that any chlorine which diffused into the flint filling reacted with methane before reaching the iron shell. The operating temperature suggested was 400-500°. In an example (93) chlorine was mixed with five times its volume of methane at ordinary temperature, while ten volumes of methane was heated separately to 370°, and mixed with the cold chlorine-methane mixture. Methyl chloride and a small amount of dichloromethane were produced.

According to Carter and Coxe (31) the more highly chlorinated products of methane were produced by passing a mixture of methyl chloride, methane, and chlorine through a reaction chamber kept at a high temperature, usually about 400-500°. The limiting temperatures for the reaction were stated to be from 350° to somewhat below 800°.

The reaction was started by heating a mixture of the three substances. It was kept in operation by continuously adding to the system chlorine and methane in the volume ratio of 2:1. From the reaction products the dichloromethane, chloroform, and carbon tetrachloride were removed by condensation; the methyl chloride and residual methane were recirculated with the added gases. The product removed contained about 80 per cent of dichloromethane, 15 per cent of chloroform, and 5 per cent of carbon tetrachloride.

In a patent of Payne and Montgomery (123) one volume of moist hydrocarbon gas—still gas, natural gas, or a mixture—was brought into contact with three or more volumes of chlorine at 150° in the presence of porous solid material made by chlorinating (122) a light paraffinic oil until it ceased to evolve hydrogen chloride. The gas was then run in, and the vessel illuminated. It was claimed that even with the high proportion of chlorine

used and the large amounts of higher paraffins and unsaturated hydrocarbons in the gas treated, practically no solid compounds, such as hexachloroethane, were formed.

Catalytic chlorination. The chlorination of methane has been carried out in the presence of a variety of catalysts, including salts, notably halides, metals, and carbonaceous materials. The results obtained, however, cannot be attributed solely to the catalysts, since most of the chlorinations have been carried out at temperatures of 300° and above, where heat alone can cause reaction. No systematic work has been reported showing accurately the effect these catalysts have on the course or speed of the chemical action.

(a) *Metallic salts and metals.* Halides of the metals have been employed more than any other class of catalytic substances to facilitate the chlorination of methane or natural gas. The substances which have been used are ferric chloride, silver chloride, "partially reduced" cupric chloride, aluminum chloride, manganese chloride, antimony pentachloride, molybdenum pentachloride, coke impregnated with the chlorides of platinum, zinc, cadmium, tin, and lead, and nickel nitrate. In patents the use of alkaline earth chlorides, zinc chloride, and thallic chloride is claimed; and sodium, cadmium, zinc, arsenic, or phosphorus in vapor form, are specified as chlorination catalysts.

(1) Methane. Pfeifer, Mauthner and Reitlinger (127) reported a series of experiments on the chlorination of nearly pure methane in the presence of antimony pentachloride and ferric chloride. The hydrocarbon was free from olefins and thoroughly dry, but contained small amounts of oxygen and nitrogen. It was mixed with chlorine and reacted by passage through a quartz tube heated in an electric oven at 360–400°. The gaseous products of the reaction were passed through two spiral condensers (the first cooled by water and the second by alcohol and solid carbon dioxide at a temperature of –35° to –45°). The distillate which collected in the first receiver consisted of dichloromethane, chloroform, and carbon tetrachloride, while that in the second receiver was mainly methyl chloride. The results are summarized in table 1.

In the presence of ferric chloride, with methane and chlorine in the volume ratio of 2:1, 85 per cent of the chlorine entered the reaction and gave 30.1 per cent of the possible yield of methyl chloride calculated on the basis of chlorine. Dichloromethane and chloroform were also formed in about equal amounts. By increasing the methane-chlorine ratio to 3:1, the yield of methyl chloride was brought up to 40 per cent of the theoretical, calculated on the basis of chlorine used.

To obtain carbon tetrachloride as the chief product, a large excess of chlorine was necessary. But since the use of more chlorine than methane when the gases were mixed by themselves caused explosion, the chlorine was diluted with nitrogen. With a gas mixture of one volume of methane, two of nitrogen, and three

TABLE I
Catalytic chlorination of methane
(Pfeifer, Mauthner and Reitlinger)

CATALYST	RATIO OF CH ₄ TO Cl ₂	CHLORINE REACTING per cent	CHLORINE IN PRODUCTS PER CENT OF TOTAL USED			
			CH ₃ Cl	CH ₂ Cl ₂	CHCl ₃	CCl ₄
SbCl ₃	1:2	64	0	5.8	20.3	5.9
FeCl ₃	1:2	83.6	Trace	13.25	22.2	6.35
FeCl ₃	1:1	86.4	5.8	15.7	21.7	0

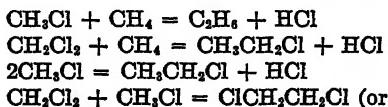
of chlorine, carbon tetrachloride and chloroform only were obtained.

Schleede and Luckow (141) passed mixtures of methane and chlorine through quartz or glass tubes (electrically heated at about 400°) with and without catalysts. When the gases were mixed in molecular proportions, inflammation occurred at first, but the flame gradually died out. The flame remained when an excess of chlorine was used. In an excess of methane ignition was not observed, but the chlorine was utilized quantitatively only if the temperature was below a certain value, which depended on the composition of the mixture and its rate of flow. These investigators obtained all the chloromethanes and, in addition, considerable amounts of chlorine derivatives of higher

hydrocarbons. If the rate of flow was considerably below that causing the ignition, an increased production of chloroform, carbon tetrachloride, or substances of similar boiling point, at the expense of methyl chloride and dichloromethane, was observed.

The results were not modified appreciably when the minimum reaction temperature was exceeded by as much as 100°, when the heating zone was increased or diminished, or when ferric chloride, molybdenum pentachloride, or antimony pentachloride were present as catalysts. The relative proportions of the chlorinated methanes were not those to be expected according to the relationship of Martin and Fuchs (108).

These workers considered that the most probable reason for the difference was that the reactions followed a route different from the step-wise series of substitutions usually postulated. Methane might first dissociate, and chlorine then react with the fragment. Methane might also react with a chlorinated derivative to give a homolog according to the following equations:



This explanation would account for the presence of halogen derivatives of the higher paraffins among the reaction products.

Tanaka (178) observed silver chloride to be an effective catalyst for the chlorination of methane in the presence of ultra-violet light.

In Boswell and McLaughlin's (21) study on the optimum yield of methyl chloride, 80 per cent or more, on the basis of the chlorine used, was obtained when moist nitrogen, methane, and chlorine in the volume ratios 70:7:1 were passed at 450° over pumice impregnated with cupric chloride. The catalyst was previously "reduced about three-quarters of the way to the cuprous condition" by nine hours heating at 450° in a current of moist nitrogen. From 10 to 15 per cent of the chlorine went to form dichloromethane, but the formation of chloroform or carbon tetrachloride was negligible.

Carbon tetrachloride in 90 per cent yield, free from other chloromethanes, was obtained by passing methane and chlorine in

approximately theoretical proportions (1:4) over a fresh sample of the cupric chloride catalyst prepared as stated.

(2) Patents. Pfeifer and Mauthner (126) obtain all the chlorine derivatives of methane by causing mixtures of chlorine and this hydrocarbon, proportioned according to the product desired, to react at a temperature of 150–500° in the presence of free metals or salts of metals which exhibit two valencies, such as iron, copper, or antimony.

The Holzverkohlungs Industrie A.-G. and Roka (64, 66, 137) used as catalysts cupric chloride, ferric chloride, and halides of the alkaline earth metals, particularly calcium or magnesium chloride, employing the salts either alone or diluted with a porous carrier. They used steam as a diluent to moderate the violence of the reaction and at the same time to supply, when necessary, heat for its initiation. From two to five volumes of steam per volume of chlorine were mixed with sufficient methane to give the products desired. The reaction temperature was kept between 100° and 650°, being usually about 400–500°. Other diluents (68, 69) recommended were hydrogen chloride, carbon dioxide, and nitrogen.

According to another patent (67) of the same firm, methane and chlorine were passed into a liquid medium of high boiling point,—for instance, fused zinc chloride or potassium bisulfate, or mixtures of the chlorides of the alkali or alkaline earth metals of suitable melting point. The chlorination was aided by addition of catalysts such as ferric chloride or cupric chloride. The gases were mixed without a diluent and were kept below the temperature of reaction until they entered the fused medium. With proper regulation of the gas stream and use of suitable reaction vessels to prevent local overheating, the reaction proceeded quietly and gave good yields. From one mole of chlorine and two moles of methane, passed into a reaction vessel containing potassium bisulfate at 320°, about half of the theoretical yield of methyl chloride was obtained, together with relatively small quantities of dichloromethane and chloroform.

Polányi and von Bogdandy (131) claimed to produce methyl chloride by adding a small amount of sodium, cadmium, or zinc

in vapor form to a mixture of methane and chlorine. The use of arsenic and phosphorus (132) was reported in another patent. It was assumed that the reaction was due to the liberation of atomic chlorine by the action of the metal vapor.

(3) Natural gas. Tolloczko and Kling (183) chlorinated natural gas at as low a temperature as possible in the presence of pumice, alone or impregnated with cupric or manganous chloride. In experiments with an excess of chlorine, a Jena glass tube 1 meter long was filled with pumice and heated to 400° in an electric furnace. At a gas velocity of 2.5 liters per hour, the yield of chlorinated material was about two grams per liter. The chlorinated products were separated by distillation into two main fractions, one boiling from 75–150°, and the other above 150°. The more volatile fraction consisted of 78 per cent of carbon tetrachloride, 14 per cent of tetrachloroethane and smaller amounts of hexachlorobenzene and hexachlorobutadiene.

Natural gas was also chlorinated in the presence of pumice soaked with cupric or manganese chloride. The copper salt proved the better catalyst, allowing a considerable lowering of temperature and increase in speed of gas flow over that feasible when no catalyst was present. From two passages of the gas mixture through a tube at 300° using half as much chlorine as natural gas, and passing the mixture twice through the apparatus, there was obtained, per liter of natural gas, 0.79 gram of oil and 0.35 gram of gaseous products. In the latter methyl chloride and dichloromethane predominated, with a little ethyl chloride also present. The oil contained chloroform, carbon tetrachloride, tetrachloroethylene, and acetylene tetrachloride, and in the fraction boiling above 165°, dichloroethane. The presence of its higher homologs was thought to induce the chlorination of methane, since only a slight reaction was said to take place when the pure hydrocarbon was used in place of natural gas.

Garner (53) reported that 1000 cubic feet of natural gas when passed through a tube containing clay, pumice, infusorial earth, or aluminum chloride as catalyst at a temperature of 270–290° and treated with 526.4 pounds of chlorine gave 51.34 pounds of carbon tetrachloride, 189.8 pounds of chloroform, 62.86 pounds of

dichloromethane and 13.06 pounds of methyl chloride. With metallic iron or ferric chloride as catalyst both dichloromethane and methyl chloride could easily be converted into chloroform and carbon tetrachloride.

Gault and Benlian (55) have published preliminary results on the chlorination of natural gas of Vaux, France. Equal volumes of the gas and chlorine were passed over a ferric chloride catalyst in the dark at temperatures ranging from 245–415°. They obtained a mixture of the four chloromethanes.

Jones, Allison and Meighan (75, 76) chlorinated Pittsburgh natural gas, which contained 89.5 per cent of methane, 10.1 per cent of ethane, and 0.4 per cent of nitrogen, by passing mixtures with chlorine over such catalysts as metals, salts, and carbon in a glass tube heated in an electric furnace. The reaction began when the catalyst temperature was slightly below 250°. At this temperature the chlorine reacted with the ethane in preference to methane. The most suitable temperature found was 400°. After the reaction had started, very little application of heat was necessary to maintain the temperature. The greater the rate of gas flow, the less the heating required.

If the temperature of the reaction chamber rose above 500°, the velocity became explosive and the only products were carbon and hydrogen chloride. These investigators stated that "the heat control of the reaction is the important phase of the problem that must be solved before the work can be placed on a plant basis."

The catalysts most suitable for chloroform production were coke, impregnated with nickel nitrate or with chlorides of platinum, zinc, cadmium, iron, or tin. Using platinized coke, 50 per cent of the natural gas was converted into chloroform and 34 per cent into other chlorinated products. The highest yield of methyl chloride, 31 per cent of that possible on the basis of the natural gas used, was obtained by the use of two volumes of chlorine to one of natural gas in the presence of cokes impregnated with nickel, tin, or lead. Addition to the gas of a small amount of moisture, up to about 1 per cent, was said to promote the chlorination, although the data reported showed no decided effect.

(b) *Carbonaceous materials.* Activated charcoal, animal charcoal, and animal charcoal mixed with finely divided calcium oxide have been used in methane and natural gas chlorination, at temperatures up to 400°. The effectiveness of these materials appears due in part, at least, to their ability to absorb large quantities of chlorine.

(1) Methane. Schleede and Luckow (141) observed that the production of carbon tetrachloride and of highly chlorinated derivatives of higher hydrocarbons from methane at 400° was increased by the use of activated charcoal.

Curme (37) also reported the chlorination of methane by passing it mixed with chlorine over activated charcoal. The proportion of chlorine, which was usually from 5 to 30 per cent of the gas mixture, was varied according to the product desired.

Huff (70) pointed out that in the chlorination of methane in the presence of a charcoal catalyst the absorption of the heat developed presents difficulty because of the high reaction heat and the low thermal conductivity of charcoal. As a possible solution of this problem, he suggested that the catalyst be mounted upon the inside of a reaction tube surrounded by a liquid of high heat capacity kept in motion, or that use be made of the latent heat of vaporization of an atomized liquid carried into the reaction chamber by the gas. He favored, however, making the contact time of the gas with the catalyst brief, and alternating with cooling periods. He employed a short electrically heated contact grid to hold the catalyst.

A process of Mallet (107) carried out the reaction of chlorine with methane at a temperature between 30° and 90° in the presence of animal charcoal or other porous material, to give chloroform, dichloromethane, and carbon tetrachloride.

According to Yoneyama and Ban (197) methyl chloride was prepared easily without explosion by passing dry methane and chlorine at the respective rates of 3 and 5.5 liters per minute, over a catalyst composed of a mixture of animal charcoal and finely divided calcium oxide at 250°.

(2) Natural gas. Phillips (130) caused natural gas (containing 95.4 per cent of methane) and chlorine to react in glass combus-

tion tubes, empty or containing bone-black, sand, or asbestos. Usually the tubes were heated to the lowest temperature which would cause the color of the chlorine to disappear. Phillips found that the tendency was always toward the formation of methyl chloride and carbon tetrachloride, and that dichloromethane and chloroform were normally produced in relatively small quantities. He believed, therefore, that the manufacture of chloroform from natural gas would prove difficult.

At about 400° and using a large excess of chlorine and activated charcoals or "batchite" (98) (a material made from anthracite), Jones, Allison and Meighan (76) also obtained from natural gas large amounts of carbon tetrachloride and hexachloroethane, and relatively small quantities of chloroform.

MacKaye (105, 106) claimed to be able to produce chlorohydrocarbons, including chloroform and carbon tetrachloride, continuously on a commercial scale, by mixing natural gas or methane and chlorine at a controlled temperature in a chamber containing granulated porous material such as coke, which had been treated to remove its sulfur and iron content. The mixture was then drawn into a reaction zone also containing this catalyst material, where it was subjected to a temperature of between 250° and 600°, depending upon the speed of gas flow and the relative purity of the reactants. The proportions of methane and chlorine and the temperature of the reaction chamber were adjusted according to the product desired. Cooling means were provided to prevent the return of reaction products into the mixing chamber.

Garner and Clayton (54) mixed natural gas with chlorine at room temperature in the first half of a reaction chamber filled with freshly calcined vegetable or animal charcoal, and then passed the gaseous mixture over more of the catalyst in the second section of the chamber, heated to about 300°. In an example, 50 volumes of natural gas containing 84.7 per cent of methane, 9.4 per cent of ethane, 3.0 per cent of propane, and smaller amounts of other paraffins were allowed to react with 33 volumes of chlorine. All of the chloromethanes and derivatives of ethane as well were obtained.

Sper (175) covered very indefinitely the chlorination of saturated hydrocarbons, including methane and ethane, by mixing them with sufficient chlorine to produce the desired derivative and passing the mixture through a reaction chamber filled with small pieces of graphite and maintained at "the temperature necessary for the reaction."

(c) *With chemical chlorinating agents.* A few patents cover the chlorination of methane by reagents which liberate chlorine. Phosgene, sulfonyl chloride, or antimony pentachloride have been mixed with the hydrocarbon and the mixtures exposed to temperatures within the range of 250–450°, producing chiefly methyl chloride. Cupric chloride alone and hydrochloric acid in the presence of oxygen are also used similarly.

For the preparation of methyl chloride Hochstetter (63) proposed to conduct a mixture of methane and phosgene, in the volume ratio 3:2, over finely divided charcoal in porcelain tubes maintained at 400°. The reaction product was cooled, passed through water to absorb the hydrogen chloride, and the methyl chloride condensed by cooling under pressure.

Electrochemische Werke, Bosshard, Steinitz und Strauss (44) pass sulfonyl chloride and methane mixed in the ratio of 16:135 by weight, through an iron tube containing a catalyst, such as charcoal, metals, or metal halides, at a temperature of 300–450°. It is stated that the substances produced can be separated easily: hydrogen chloride and sulphur dioxide may be removed by absorption in water, chloroform and carbon tetrachloride by condensation at ordinary pressure, and methyl chloride and dichloromethane by condensation under increased pressure. By this process a conversion of 30 to 35 per cent of the methane used into methyl chloride, and 15 to 20 per cent into a mixture of dichloromethane and chloroform, is claimed.

The Holzverkohlungs-Industrie A.-G. (65, 85) allows methane to react with antimony pentachloride at temperatures of 300–350° in the presence of refractory clay or pumice to aid contact of the two substances. The product is largely methyl chloride with small amounts of dichloromethane and chloroform. The antimony trichloride formed during the chlorination is condensed,

rechlorinated, and returned to the reaction chamber. The use of catalysts permits the temperature to be lowered to 250°. Among the catalysts usable are the chlorides (85) of iron, copper, or calcium, preferably precipitated upon substances presenting a large surface, such as charcoal, refractory clay, or pumice.

Krause and Roka (86) reported the chlorination of methane, alone or mixed with other gases, by treating it with a mixture of hydrogen chloride, steam, and oxygen at temperatures between 300° and 650°, preferably at 450–550°. Reaction was promoted by carbon, cupric chloride, ferric chloride, thallic chloride, the chlorides of the alkaline earths and of the rare earth metals. From 60 to 80 per cent of the chlorine of the acid used appeared in the chloromethanes produced.

The I. G. Farbenindustrie A.-G. (71) also claimed to produce chloromethanes by passing a mixture of methane, oxygen, and hydrogen chloride over cupric chloride at 300–450°.

Tizard, Chapman and Taylor (181) passed methane over cupric chloride heated at 430–500°. When the halide became inactive through conversion to cuprous chloride it was regenerated by treatment with chlorine. The four chloromethanes were formed in proportions depending upon the temperature employed and the rate of flow of hydrocarbon. Slow flow and high temperature favored the formation of carbon tetrachloride and chloroform.

Electrical chlorination. The silent electrical discharge and electric sparks have been used to some extent as activating agents in the chlorination of methane or natural gas.

(a) *Methane.* Pfeifer and Szarvasy (128, 177) proposed in a patent the employment of the silent discharge with methane. Either chlorine or a substance which would liberate the halogen (128) in the discharge space was used and the discharge was sometimes aided by light, heat, or catalysts. Under this treatment a mixture of equal volumes of methane and chlorine was reported to produce all the chloromethanes.

(b) *Natural gas.* Baskerville and Riederer (12) observed that a mixture of chlorine and natural gas, saturated with water, burned continuously when directed into a spark between carbon

terminals, but the yield of chlorinated products obtained was too small for their identification. According to a patent of Colin (36) methyl chloride, dichloromethane, and chloroform were made by burning natural gas and chlorine in a reaction chamber through which an electric spark was passing. One volume of chlorine was used for each atom of hydrogen which it was desired to replace. If chloroform was to be produced the reaction chamber was kept at a temperature of 100–200°. In the production of methyl chloride the chamber was cooled by passage of air around it, which prevented the formation of carbon tetrachloride. This process (11) went as far as an unsuccessful experimental plant at Allegheny, Pennsylvania.

2. Ethane

Ethane is reported to react with chlorine more readily than methane. It is stated that ethane will chlorinate in a natural gas containing but 10 per cent of this hydrocarbon with scarcely any formation of chloromethanes. Like its lower homolog, however, ethane does not change when in contact with chlorine in the dark at ordinary temperatures. To bring about reaction, diffused daylight and artificial light have been used. Catalysts, notably charcoal and cupric chloride, have found some use, particularly in the production of chloroethanes from natural gas.

From the reaction of pure ethane and chlorine only two products, ethyl chloride and dichloroethane have been identified. From natural gas a trichloroethane, a tetrachloroethane, and hexachloroethane have also been obtained.

Photochemical chlorination. Kolbe and Frankland (83) employed chlorination in their early work on the structures of ethane and other paraffin hydrocarbons. When dry ethane was mixed with an equal volume of chlorine in the dark, and the mixture exposed to diffused daylight, ethyl chloride was produced. By the reaction of two volumes of chlorine with one of ethane (51) some ethylene dichloride was obtained. A substance resembling camphor (83) resulted when ethyl chloride mixed with a large amount of chlorine was exposed to sunlight.

Schorlemmer (146) found that ethane, obtained either by

electrolysis of potassium acetate or by the action of strong sulfuric acid on mercury diethyl, whether dry or moist (147, 148) gave the same products when mixed with an equal volume of chlorine in the dark and then exposed to diffused daylight at 5°. These products were chiefly ethyl chloride, with a small amount of ethylene dichloride. Schorlemmer used this fact to prove that the hydrocarbons from these two sources were identical.

Darling (38) also obtained ethyl chloride and ethylene dichloride by exposing equal volumes of chlorine and ethane, mixed in the dark, to diffused daylight.

In the treatment of natural gas with an excess of chlorine in the presence of light from an ultra-violet lamp, Tolloczko (182) obtained, as has already been noted, hexachloroethane and an oily product which was not identified.

The chlorination of ethane and higher paraffins in the presence of artificially produced light rich in chemically active rays, has been claimed in a patent of the Badische Anilin und Soda Fabrik (7). Graul and Hanschke (57) chlorinated mixtures of chlorine with ethane and higher hydrocarbons by exposing them to light from a mercury-vapor lamp.

Tompkins (184) claimed the preparation of chloroethanes from ethane, obtained from coal gas by liquefaction, with the aid of actinic light. He also found it possible to chlorinate the crude oil gas, containing about 20 per cent of ethane, by the use of actinic light of gradually increasing intensity, so that the chlorination of methane was avoided, and only derivatives of ethane were formed. The intensity of the light was controlled either by passing the gas through the apparatus so arranged that the distance of the gas from the light source was gradually diminished, or so that it was screened from the light by paper or other translucent material of gradually diminishing thickness.

Snelling (168) chlorinated ethane, as he had chlorinated methane, in an illuminated chamber provided with transparent baffles, using an excess (173) of the hydrocarbon and circulating the gas mixture from the reaction vessel into contact with a solvent for the halogenated products, such as oil or carbon tetrachloride.

Thermal chlorination. When using a reaction vessel of silica, earthenware, or brick, maintained at a temperature of from 300° to 550°, Lacy (90) claimed that a large excess of hydrocarbon, such as eight volumes of ethane to one of chlorine, restricted the product to monochloroethanes. According to another method (91) the dried reaction product containing crude ethyl chloride was passed over calcium oxide at 250° to 450° to produce ether.

Catalytic chlorination. In most of the published work on the catalytic chlorination of ethane, charcoal catalysts have been used. In the presence of war-gas charcoal, Jones, Allison and Meighan (75, 76) found that the ethane present in natural gas (10 per cent of ethane with 90 per cent of methane) was chlorinated in preference to the methane and at a lower temperature. Hexachloroethane was the chief product, with some ethyl chloride, dichloro-, trichloro-, and tetrachloro-ethane.

Mallet (107) mixed ethane and chlorine and passed them through a layer of animal charcoal or other porous contact material at a temperature between 30° and 90°. Curme (37) chlorinated ethane, among other hydrocarbons, by contact with chlorine in the presence of activated charcoal. Garner and Clayton (54) proposed the formation of chloroethanes by passing mixtures of natural gas and chlorine through charcoal at 300°.

Tolloczko and Kling (183), by chlorinating natural gas in the presence of pumice, or pumice impregnated with cupric chloride at 400°, produced hexachloroethane when chlorine was in excess, and ethyl chloride, dichloro-, and tetrachloro-ethane when an excess of natural gas was used.

Electrical chlorination. A mixture of ethane and chlorine is exposed to the influence of the silent electric discharge in a modification (8) of a process patented earlier by the Badische Anilin und Soda Fabrik (7).

3. Propane

This hydrocarbon, like methane and ethane, is not acted upon by chlorine in the dark, but from experiments with mixtures it appears to be more readily chlorinated than methane. In sunlight, *n*-propyl chloride, 1,2-dichloropropane, and more highly

chlorinated products may be obtained, the extent of the reaction depending upon the intensity of the light and the time of exposure. Under extreme conditions, such as the use of iodine chloride at 250°, chlorinating agents can break down the hydrocarbon structure, producing halogen derivatives of lower carbon content.

In the study of the structure of propane, Schorlemmer (152) investigated the chlorination of this hydrocarbon. He found that a mixture of chlorine with slightly more than an equimolecular proportion of propane reacted in diffused daylight, and yielded a product consisting of a low-boiling fraction which was 1,2-dichloropropane with small quantities of *n*-propyl chloride, and a mixture of more highly chlorinated products boiling between 100° and 200°. To effect further chlorination, a considerable amount of the fraction boiling above 80°, mainly dichloropropane and higher products, was exposed to direct sunlight while chlorine was passed into it for several days. After this treatment the greater portion of the product boiled between 150° and 160°. While it was impossible to isolate a fraction having a constant boiling point, the chemical behavior, boiling point range, and chlorine content of this fraction showed that it consisted of trichloropropane mixed with more highly chlorinated products.

The residual liquid, after further chlorination in direct sunlight for several days, was composed partly of a liquid and partly of a fraction boiling between 200° and 205° which solidified in the receiver as a white, crystalline mass, which after recrystallization had the chlorine content of tetrachloropropane. The liquid from this chlorination, which boiled between 205° and 250°, was acted upon very little by chlorine even in the brightest sunlight in the presence of iodine. Treatment with potassium chlorate and fuming hydrochloric acid also had little immediate effect, but by exposure to these reagents for several days, the boiling range of the liquid was raised to between 220° and 250°. From this liquid hexachloropropane was obtained, a substance which had a camphor-like odor and boiled at about 250°.

Mereshkowsky (111) reported the formation of tetra-, penta-, and hexa-bromides by the action of bromine on propane in the presence of iron, but gave no experimental details.

Snelling (167, 168) chlorinated propane in the reaction chamber provided with transparent baffles, already described, through which a stream of mixed gases passed toward a source of actinic light. The products (173) were removed from the reaction vessel, as in the case of the other hydrocarbons chlorinated in this way, by a solvent in which the unchanged hydrocarbon was sparingly soluble.

Herzfelder (62) studied the further chlorination of seven di- and tri-chloropropanes by antimony pentachloride at varying temperatures up to 190°. He reported products as shown in table 2.

TABLE 2
Products obtained by chlorination of chloropropanes by antimony pentachloride

INITIAL SUBSTANCE	PRODUCT
1,1-Dichloropropane	1,1,2-Trichloropropane
1,2-Dichloropropane	1,1,2-Trichloropropane
1,3-Dichloropropane	1,1,3-Trichloropropane
2,2-Dichloropropane	1,2,2-Trichloropropane
1,2,3-Trichloropropane	1,1,2,3-Tetrachloropropane
1,2,2-Trichloropropane	1,2,2,3-Tetrachloropropane
1,1,2-Trichloropropane	Pentachloropropane

4. Butanes

n-Butane. A number of studies have been made of the chlorination of butane, which, like its lower homologs, does not react with chlorine in the dark, but readily undergoes reaction in sunlight or artificial illumination. The only products that have been identified are a butyl chloride and a dichlorobutane.

Eighty years ago, Frankland (50) observed that a dry mixture of chlorine with butane reacted immediately on exposure to diffused daylight. Butane did not react with antimony pentachloride even in direct sunlight.

Schöyen (162) found that chlorine and butane did not react in the dark, but when butane over water in a flask of white glass was quickly mixed with an equal volume of chlorine, and the flask placed in bright daylight, reaction took place immediately

with the formation of hydrochloric acid and a liquid insoluble in water, which was a mixture of butyl chloride, butylene chloride, and a more highly chlorinated liquid. Even in very weak sunlight reaction occurred.

Ronalds (139) found that butyl chloride was formed when butane from American petroleum reacted with two volumes of chlorine in the presence of light.

From ten volumes of butane and nine volumes of chlorine, exposed to daylight, Butlerov (26, 27) obtained a product containing somewhat more chlorine than called for by the formula $C_4H_9Cl_2$.

Patents owned by the Badische Anilin und Soda Fabrik (57, 73) cover the production of chlorine derivatives from butane and the other low-boiling paraffin hydrocarbons such as pentane, hexane, and heptane in the vapor phase in the presence of ultra-violet light. Snelling (173) mixed chlorine with an excess of butane in a reaction vessel from which the gaseous mixture could be circulated and brought into contact with oil or carbon tetrachloride, as a solvent for the halogenated products, and a second solvent to remove hydrogen chloride.

Butyl chloride was one of the halogen derivatives obtained by Brooks, Essex and Smith (24) by chlorination of a gasoline fraction boiling between 25° and 45° in the presence of actinic light.

Mabery and Hudson (103) chlorinated a number of petroleum fractions boiling within the range -10° to 20°, but in no case did they isolate *n*-butyl chloride.

A petroleum distillate boiling between 5° and 10° which Pelouze and Cahours (124) treated with chlorine in diffused light, yielded a product which boiled between 65° and 70° and possessed the properties and composition of butyl chloride.

Isobutane. Only a few studies have been made of the chlorination of pure isobutane. In bright sunlight the reaction is violent and explosive; in diffused light it can be controlled and yields both monohalides and more highly chlorinated products. Work on petroleum fractions has also yielded isobutyl halides.

By exposing a mixture of ten volumes of isobutane and nine of chlorine to the action of daylight, Butlerov (26) produced

tertiary butyl chloride and a chlorinated liquid very similar to that which he had obtained by the same method from butane. It contained slightly more chlorine than does a dichlorobutane.

A naphtha fraction boiling from -2° to 2° , from Pennsylvania and Ohio petroleum, was observed by Mabery and Hudson (103) to react violently with chlorine in bright sunlight with the appearance of a flame and separation of carbon. To obtain smooth reaction and highest yield of monochloro derivative, they worked in the cold with a sheet of newspaper interposed between the reaction vessel and the sun. Chlorine was slowly passed into a 2-liter bottle which contained from 100 to 200 cc. of oil. The delivery tube for introduction of halogen extended half way from the top of the bottle to the surface of the liquid. Continuous operation during 10 to 12 days was required to chlorinate 100 grams of naphtha. The reaction was stopped while a small amount of the unchanged hydrocarbon remained. After twenty fractionations, 80 per cent of the product was collected at $68-69^{\circ}$, and by reason of the properties of the acetate, alcohol, and sulfide into which it was converted it was thought to be an isobutyl chloride. By prolonged fractional distillation one other chlorobutane was obtained, which distilled at $121-122^{\circ}$ and was shown by analysis to be a dichlorobutane.

Aschan (3) chlorinated in the presence of water a petroleum fraction which boiled at $10-12^{\circ}$. The formation of a chloride, C_4H_7Cl , with a boiling range of $60-70^{\circ}$, as the main product, indicated that butanes were among its components. The presence of isobutane was established; *n*-butane appeared to be either absent or present in only small quantity.

Without describing any details of his experiment, Mereshkowsky (111) stated that he formed tetra-, penta-, and hexa-bromides by the action of bromine on isobutane in the presence of iron.

Krafft and Merz (84) reported that heating isobutane with iodine chloride at 250° caused decomposition of the hydrocarbon and yielded the same products as did similar treatment of propane, namely, hexachloroethane and carbon tetrachloride.

In the combustion of isobutane in a mixture of oxygen and chlorine, Schlegel (142) observed that the hydrogen combined preferentially with chlorine and the carbon with oxygen.

5. *Pentanes*

The work done on chlorination of pentanes has as a rule not been carried out on the normal hydrocarbon or on an isomer in pure form, but on "pentane" fractions from petroleum, some sufficiently closely fractionated to be pentane or isopentane in nearly pure form, others boiling over a considerable range and probably containing all three pentanes and perhaps higher hydrocarbons as well. The chlorination of the fractions has had commercial success, and is a source of amyl chlorides, from which the alcohols, acetates, and other derivatives can be made.

From pure *n*-pentane have been obtained *primary* and *secondary* amyl chlorides and unidentified more highly chlorinated substances. Isopentane appears to chlorinate more easily than the normal hydrocarbon. As products from isopentane all of the four possible monochlorides have been identified. Mixed pentane fractions, which have usually been chlorinated at elevated temperature, and often in the presence of light and catalysts, have yielded chiefly monochloropentanes, the reaction normally being controlled so as to avoid the formation of higher products. In several studies mention is made of the fact that the presence of moisture aided chlorination.

n-*Pentane*. Cahours and Demarcay (28) found that pentane (boiling at 32–35°, whereas the accepted boiling point of pentane is 36.2°), separated from an oil produced by distilling crude fatty acids with superheated steam, absorbed chlorine rapidly in diffused light. When the action was not continued too long a liquid was produced which boiled at about 100° and had the characteristics of amyl chloride.

Lachowicz (87) obtained two monochloropentanes by passing a slow stream of chlorine into the vapors of *n*-pentane at room temperature. The retort in which the reaction took place was provided with a well-cooled reflux condenser. After the reaction had started it was accelerated by heating to boiling on a water bath. Two amyl chlorides were formed, the one which was formed in larger quantity boiling at 106°, and the other, apparently a *secondary* chloride, boiling at 104°.

When Schorlemmer (156) passed a strong current of chlorine

into the vapors of a petroleum fraction boiling at 37–39°, he obtained a mixture of amyl chlorides boiling between 95° and 110°; the main fraction distilled at 100–102°.

Aschan (3) identified 1-chloropentane and secured a second amyl chloride which boiled at 96–98° from petroleum pentane. The best results were obtained when the hydrocarbon was thoroughly dry during chlorination.

The Badische Anilin und Soda Fabrik (5) raised pentane to its boiling point in an enamelled vessel provided with a fractionating column, mixed the vapors with chlorine, and subjected them at 40–50° to the influence of the silent electric discharge. They recommended that two volumes of vapor be mixed with one volume of chlorine. Diminished pressure (9) was said to promote the action.

Bauer (13) found that when chlorine was passed for several hours into pentane a product was formed which boiled between 230° and 240° and to which he gave the formula $C_5H_8Cl_4$. The pentane absorbed chlorine and evolved hydrogen chloride so rapidly that sufficient heat was produced to raise the liquid to the boiling point.

Isopentane. Aschan (3) found that isopentane from petroleum reacted more readily with chlorine than did pentane. Chlorination of the dry hydrocarbon yielded a mixture of the four monochloroisopentanes (monochloro-2-methylbutanes) together with dichlorides. Chlorination of moist isopentane produced large amounts of the *primary* isoamyl chlorides, small quantities of *secondary* isoamyl chloride and no *tertiary* product. The best yields of monochloropentanes, however, were obtained when the isopentane was dry. Aschan also investigated the conversion of these isopentane derivatives into isoprene.

Pelouze and Cahours (124) observed that an isopentane fraction from petroleum, boiling at 30–32°, absorbed chlorine rapidly at ordinary temperature even in diffused light. By avoiding an excess of chlorine and stopping the reaction while a part of the hydrocarbon remained unchanged, they obtained an amyl chloride boiling at 98–103°.

Lachowicz (87) obtained monochloro derivatives of isopentane

by the action of a slow stream of chlorine on isopentane (b. p. 29–30°), obtained from Galician petroleum. The reaction was started at room temperature and after some of the chloride had been formed, was continued at the boiling point of the hydrocarbon. The chloroisopentanes boiled at 99.8–100.5°, and had a specific gravity of 0.8703 at 20°.

Mabery and Hudson (103) exposed several hundred grams of a distillate of boiling point 29–30°, obtained from Pennsylvania and Ohio petroleum, to the action of chlorine until they obtained about 200 cc. of substitution products. As in the chlorination of butane, the reaction was carried out in the presence of sunlight subdued by passing through newspaper. After eighteen distillations of the chlorinated products about 85 per cent of it boiled at 96.5° and was proved to be isoamyl chloride.

The only other constant boiling fraction that could be obtained from the chlorination products was collected at 144–145° after fifteen distillations. It amounted to about 5 grams, and had the composition of a dichloropentane.

Mott and Bedford (119) observed that at room temperature mixtures of chlorine and isopentane reacted twice as rapidly when exposed to light from a white flame arc as when exposed to that from a quartz mercury-vapor lamp. The white flame arc was rich in blue light, which has been shown to be more suitable than ultra-violet for chlorination.

According to patents controlled by Badische Anilin und Soda Fabrik (6, 74) a mixture of chlorine and an excess of isopentane is exposed to radiation from a mercury-vapor lamp. A mixture of two monochloroisopentanes is obtained from which isoprene may be produced. A process apparently identical with that of the Badische Company (74) is outlined in a patent of Graul, Hanschke and Webel (58).

According to a patent of the Chemische Fabrik auf Aktien (32), a mixture of four isoamyl chlorides boiling between 85° and 105° was obtained by chlorinating isopentane. The Badische Anilin und Soda Fabrik (9) prepares dichloropentane from isopentane under diminished pressure.

Tetramethylmethane. Tissier (180) obtained the monochloro

derivative by allowing a mixture of chlorine and this hydrocarbon to stand in ordinary light at room temperature.

Pentane mixtures (petroleum fractions). (a) *Photochemical chlorination.* Brooks, Essex and Smith (24, 25) prepared amyl chlorides from a pentane fraction (boiling range 25–45°) from petroleum. Chlorine was introduced into the hydrocarbon material in the dark at a temperature just sufficient to vaporize it completely. The resulting mixture was then passed through glass tubes illuminated by an ultra-violet lamp. Once started, the chlorination proceeded smoothly in diffused daylight and the lamp was unnecessary. The highest yields of monochloro derivatives were obtained when the chlorine-hydrocarbon mixture contained a large excess of hydrocarbons, namely, 2 to 4 times the ratio necessary to form the monochloro compounds. When employing mixtures containing three moles of hydrocarbon to one of chlorine, amyl chlorides made up 88 per cent of the chlorinated product. They had difficulty in regulating the ratio of hydrocarbon vapors to the chlorine introduced, and for this reason obtained the formation of dichloro and trichloro derivatives as well. The hydrogen chloride formed in the reaction was absorbed in water to prevent loss of pentane vapor.

When chlorinating in the liquid phase, 90 to 94 per cent of the chlorinated product consists of monochloropentanes. The chlorine was passed through a large number of small orifices into cold pentane, and the chlorination stopped before the concentration of chlorine derivatives became greater than about 20 per cent of the mixture. The process was controlled by observing the specific gravity of the mixture. The presence of moisture seemed to aid the chlorination. Although illumination of the reaction mixture was not absolutely necessary, at the start these workers used light from a high-power tungsten bulb in the upper part of the reaction chamber. In order to prevent excessive vaporization of pentane, the temperature was not allowed to rise above 10°. From the chlorinated products a monochloropentane fraction suitable for conversion into amyl acetate was obtained.

About the time this information was published (1918), Gardner (53) described a similar process in which a natural gasoline frac-

tion boiling between 40° and 70° was chlorinated in the presence of ultra-violet light to produce amyl chloride.

Dobryanskii and Gurevich (40) chlorinated a pentane cut (boiling at 31–38°) from aviation gasoline in the gas phase using artificial light. The highest yield of monochloride (35 per cent) was obtained at 60–70°. Wet chlorine increased the formation of polychlorides, and also led to explosion and separation of carbon.

Sparre (174) claimed the chlorination in the presence of light of a naphtha fraction boiling between 28° and 69° and said to consist of "pentanes, isopentanes, hexanes, and isohexanes."

The patent of Boyd (22) specified the chlorination of pentane, as well as of others of the first eight paraffins above methane, by treating the hydrocarbon in the liquid phase with chlorine, and then exposing the vapors from this step to actinic light in a separate reaction chamber. Superatmospheric pressure was considered advantageous in the vapor phase stage of the chlorination.

Lacy (94) disclosed a method of chlorinating pentane and other hydrocarbons by dissolving chlorine in the hydrocarbon floating on water, and then exposing the solution to light from a quartz mercury-vapor lamp. The hydrogen chloride produced was absorbed by the water.

Patents controlled by the Badische Anilin und Soda Fabrik (57, 73) already referred to under the chlorination of butane, claim applicability to the chlorination of pentane in the presence of ultra-violet light.

(b) *Photochemical-catalytic chlorination.* As catalysts to assist the photochemical chlorination of pentane charcoal, batchite, and silica gel have been used, and, in addition, the use of ferric chloride and iron is covered by patents.

Koch and Burrell (80) found that in the vapor phase, chlorination of a pentane fraction from natural gasoline was difficult to control, and di-and tri-chloropentane were formed, as well as the desired amyl chloride. Accordingly an apparatus (79, 81) was devised in which it was possible to carry out chlorination on an industrial scale in the liquid phase at any desired rate

without danger of explosion or formation of di- or tri-chloro derivatives. The apparatus consisted of a vertical tube of lead-free glass provided at the lower end with a screen through which a chlorine-injecting nozzle projected. A tube of the same material, of smaller diameter and about half as long, with its lower end spread out in the form of a funnel, was supported within the larger tube, so that the funnel was a short distance above the bottom screen. The chlorinating catalyst, which was activated charcoal, silica gel, batchite, or other porous substances with large power of gas absorption, was placed on the bottom screen in the large tube to the depth of about three inches. In carrying out the process, the large tube was filled three-fourths full of pentane, and a regulated stream of chlorine admitted by the injector at the bottom. The chlorine passed through the layer of activated charcoal and carried a portion of the catalyst with it up through the percolator tube. While flowing upward the catalyst absorbed large amounts of chlorine.

Upon passing from the top of this tube the carbon dropped on the outside of the inner tube to the bottom of the large reaction tube. In falling it passed through a reaction zone where the absorbed chlorine reacted with the pentane under the influence of the light from the mercury-vapor lamps. The catalyst was continuously cycled while any pentane passing off as vapor was refluxed to the reaction tube.

To keep the formation of di- and tri-chloropentanes at a minimum it was necessary to stop the chlorination when the derivatives amounted to 20 to 25 per cent of the total volume of liquid. Yields of monochloropentanes were 78 per cent in actual plant practice. The residue, consisting of di- and tri-chloro derivatives, amounted to about 5 per cent.

Only 20 per cent of monochloride was obtained when Dobryanskii and Gurevich (40) chlorinated pentane from aviation gasoline in the liquid phase in the presence of activated charcoal.

In the process of Blanc (20) a stream of chlorine was introduced directly into pentane cooled by an ice bath to about 10° and exposed to daylight, sunlight, or light from a tungsten lamp, gas light, electric arc, or mercury-vapor lamp. An aqueous solution

of iron chloride was added as a halogen carrier. The presence of moisture, even in small amounts, was favorable to the chlorination. To produce dichloropentane, a slower rate of passage of the reaction mixture through the apparatus was used than that found suitable for producing monochloropentane.

Dow (41) proposed conversion of pentane, present in coal distillation products, into chlorine or bromine derivatives in order to increase its boiling point and enable the hydrocarbon to be separated from carbon disulfide.

(c) *Thermal chlorination.* The Sharples Solvents Corporation (4, 45, 52, 78) at Charleston, W. Va., produces chloropentanes on a commercial scale from a 28° to 37° fraction of natural-gas gasoline by chlorination at elevated temperatures. The process used (4), which in the patents is claimed for paraffins containing more than two carbon atoms, consists in mixing the hydrocarbon material in vapor form at about 100° with 1/15th to 1/20th part of chlorine, and passing the mixture at high velocity through a tubular reactor, such as a gas-fired pipe still (33), kept at 315° to 371°.

Clark (33) states that by the Sharples process monochloro derivatives of pentanes are produced in approximately the following proportions:

	per cent		per cent
1-Chloropentane.....	24	1-Chloro-2-methylbutane.....	30
2-Chloropentane.....	8	2-Chloro-2-methylbutane.....	5
3-Chloropentane.....	18	4-Chloro-2-methylbutane.....	15

Ayres (4) reports very similar data from the same process: "One-half of the monochloride formed from *n*-pentane is *primary* and the other half is *secondary*. Of the *secondary* fraction there is much more 3-chloropentane than 2-chloropentane. . . . In the commercial synthesis isopentane yields 85 per cent *primary* isoamyl chlorides and 15 per cent *tertiary* amyl chloride. No *secondary* isoamyl chloride is formed. (It will be noted that Aschan did obtain this substance.) Of the *primary* chlorides, one-third is 4-chloro-2-methylbutane, and two-thirds is 1-chloro-2-methylbutane." Below 100° in either vapor or liquid phase it was stated that light or catalysts are required to bring about

chlorination, and yields of *primary* chlorides are low. Use of iodine, sulfur chloride, or red phosphorus as catalysts causes excessive production of polychlorides. High monochloride yields in either liquid or vapor phase were produced when insoluble and non-volatile metallic chlorides were used as catalysts, but these materials were reported to cause transposition of *primary* to *secondary* or *tertiary* chlorides.

Dichloropentanes were identified as follows:

<i>Dichloro derivative</i>	<i>Amount present</i>
1,1-Dichloropentane.....	"Almost none"
1,2-Dichloropentane.....	"Present in minor proportion"
1,5-Dichloropentane.....	"Almost none"
2,3-Dichloropentane.....	"Major constituent"
1,2-Dichloro-2-methylbutane	"Present in minor proportion"
2,3-Dichloro-2-methylbutane	"Formed, but is mostly destroyed by polymerization."

6. Hexanes

The chlorination of hexane can be readily controlled to yield monochloro, dichloro, or more highly chlorinated products. In most of the work reported the main products have been by choice hexyl chlorides, rather than more completely substituted derivatives. The hexane has been in some cases synthetic material, but more usually a closely cut petroleum fraction has been used. Light and catalysts have been employed to a considerable extent to aid reaction. Catalysts, as a rule, encourage the formation of polysubstituted compounds. A small amount of work has been done with diisopropyl, producing monochloro derivatives.

n-Hexane. Schorlemmer (154, 155, 158) made a series of investigations of the chlorination of hexane from petroleum to determine the conditions governing the formation of the *primary* or the *secondary* chloride.

The hexane was treated with chlorine under the following conditions: (1) chlorine was passed into the well-cooled hydrocarbon in diffused daylight; (2) chlorination was carried out in the cold in the presence of iodine; (3) chlorine alone was passed into the vapors of the boiling hydrocarbon; (4) chlorine was passed into the hydrocarbon vapors in the presence of iodine. In all four

cases, the bulk of the product was a mixture of *primary* and *secondary* hexyl chlorides. When chlorine acted on liquid hexane either in the presence or absence of iodine, large quantities of more highly chlorinated products also formed, but by chlorination of the vapors, the formation of the higher halogen derivatives was avoided almost completely.

By passing chlorine into the vapor of gently boiling petroleum hexane, and fractionating the product, Schorlemmer (158) obtained monochlorohexanes which distilled at 125–126°. Chlorination of the vapor of hexane, prepared by reduction of hexyl iodide (158, 160), gave a product which distilled largely at 126–128.5°, with a smaller portion boiling at 128.5–130°. More highly chlorinated substances were also formed but in smaller quantity. "It appeared," Schorlemmer (158) stated, "that by the action of chlorine upon the hydrocarbon (hexane) one-sixth is converted into the *primary* chloride and five-sixths into the *secondary*."

Several researches made prior to that of Schorlemmer are of historical interest. Wanklyn and Erlenmeyer (190) had noted that the chlorination of hexane was aided by iodine. Riche (134) stated that hexane, obtained by treating *n*-heptoic acid with barium oxide and boiling at 58° (*n*-hexane boils at 69°), was attacked briskly by chlorine with formation of substitution products.

From American petroleum, Pelouze and Cahours (124) obtained a fraction boiling at 68° which they showed consisted essentially of hexane, and which on chlorination in diffused light yielded mainly a monochlorohexane boiling at 125–128°.

A chlorohexane with the same boiling range was formed when Cahours and Demarcay (28) chlorinated hexane (boiling point 68–70°, d_{4}^{20} 0.667) which they had separated from an oil produced by distilling crude fatty acids in the presence of superheated steam. In diffused light the continued (124) action of chlorine on the monochloride of Pelouze and Cahours formed a dichlorohexane boiling at 180–184°, density 1.087; a trichlorohexane boiling at 215–218°, density 1.193; and a tetrachlorohexane. Further chlorination with the aid of heat and sunlight yielded

a hexachlorohexane, a stable product which boiled at 285–290° and had a density of 1.598. Schorlemmer (153) also produced a hexachlorohexane by passing chlorine into hexane from petroleum as long as any action could be observed, first in diffused light and afterwards in direct sunlight. This product could not be distilled without decomposition.

Morgan (118) chlorinated hexane from petroleum (b. p. 68–70°) in the vapor phase by Schorlemmer's method (158). Nine-tenths of the hydrocarbon was converted into a mixture of monochlorides boiling at 120–134°, while the remainder formed more highly chlorinated products.

Michael and Turner (115) passed chlorine into boiling hexane in diffused daylight and obtained a mixture containing about 10 per cent of 1-chlorohexane, a small amount of 2-chlorohexane, and still less 3-chlorohexane.

The following researches are of more recent date.

Faragher and Garner (47) chlorinated isohexane and hexane fractions (boiling at 61–64°, and 67.5–70°, respectively, and obtained from Pennsylvania straight-run gasoline) in the vapor phase by the method suggested by Schorlemmer (158). They introduced the chlorine at about the middle point of a tube into which the hydrocarbon was being refluxed. Monochloro derivatives were the principal products, with small amounts of dichlorohexanes and more highly chlorinated compounds. The chlorination was carried out in diffused light, as in full daylight a flame appeared and carbon was formed. In order to obtain good yields, it was necessary to absorb the issuing hydrogen chloride or to cool it to a low temperature, so that none of the chlorinated products should be carried away and lost.

A fraction which was obtained from the chlorinated *n*-hexane, distilling at 120–124° after four fractionations, corresponded in properties to 2-chlorohexane. From isohexane was obtained a mixture of monochlorohexanes distilling at 119–123°.

According to patents controlled by the Badische Anilin und Soda Fabrik (57, 73) an excess of hexane vapor mixed with chlorine is led from below into a vessel in the middle of which a mercury-vapor lamp is placed. The vapors from this reaction vessel are

passed through a condenser where the chlorohexane and hexane are condensed and from which they are siphoned into the distillation vessel, while the hydrogen chloride escapes. Most of the chlorohexane remains in the reaction vessel until the end of a run.

Vivas (188) claims the catalytic chlorination of a purified gasoline presumably consisting mainly of hexane. The hydrocarbon material is introduced into a reaction vessel together with carbon tetrachloride, aqueous ammonia, and catalysts, such as aluminum or ferric chloride, and chlorine is bubbled through the reaction mixture. The product is said to be suitable for cleaning purposes or as an ingredient in paints.

According to the International Fireproof Products Corporation (72) a compound C_6H_7Cl , is obtained by bubbling chlorine through gasoline, consisting mainly of hexane, in the presence of aluminum chloride or ferric chloride.

2,3-Dimethylbutane (diisopropyl). Schorlemmer (151) found that diisopropyl (prepared from isopropyl iodide), with a boiling point of 58° and specific gravity of 0.6701 at 17.5° , reacted with chlorine readily in the cold. If the reaction was stopped before all the hydrocarbon had reacted, the chief product was a monochloride which boiled at 122° and had a specific gravity of 0.8874 at 22° . When iodine was present, no monochloride was formed, but the main product consisted of dichlorodiisopropyl, a solid which melted at 160° .

In the presence of iodine, Silva (165) obtained from diisopropyl both of the chlorides described by Schorlemmer (151). When the reaction was carried out in sunlight (166) two monochlorides were formed: one was that of Schorlemmer (151), the other boiled at 118° . In the absence of iodine the lower boiling monochloride and a liquid dichloride which boiled at 160° were formed.

Aschan (2) subjected diisopropyl (a $57\text{--}59^\circ$ fraction of Baku petroleum) to chlorination in a retort cooled externally with ice water and provided with reflux condenser. In order to avoid the formation of more highly chlorinated products, at frequent intervals the portion boiling above 100° was separated by distillation, and the chlorination of the remainder continued. By fractionation of the final product two liquids were obtained, one of

which boiled at 117–119° and was the *tertiary* chloride, while the other, which boiled at 123–125° was an unidentified monochloro derivative. Later Aschan (3) reported the formation of both these substances and a third monochlorohexane of undetermined structure on chlorinating a 58–69° petroleum fraction.

7. *Heptanes*

From the published work on heptane chlorination, it is apparent that the halogen and either the normal hydrocarbon or its isomers react readily in diffused daylight, or in the dark in the presence of catalysts such as iodine. Bright sunlight is likely to cause a disruptive action with separation of carbon. Some of the investigations reported have been made with synthetic heptane, but there is doubt sometimes of the purity of the material used, as reported boiling points do not agree with the accepted value. Many of the workers employed petroleum fractions, which had such wide boiling ranges that considerable amounts of hydrocarbons other than those designated must have been present.

Usually chlorination has been stopped when only monochloro derivatives were produced. There is no difficulty, however, in producing higher products by prolonging the action, especially when using catalysts. In several cases there is doubt as to the exact structure of the chloroheptanes produced. 1-Chloro- and 2-chloro-heptane have been identified, but in some investigations the workers have been content to simply report the production of monochloroheptanes and to give boiling points and densities.

n-Heptane. In one of the early studies of paraffin hydrocarbons Schorlemmer (143) observed that when chlorine was passed into heptane, "hydride of heptyl," the liquid became warm and hydrogen chloride was evolved. From the products of the reaction he isolated a monochloride which boiled at 150–152° and had a specific gravity of 0.891 at 19°. Smaller amounts of higher boiling chlorine compounds were formed, but they were not identified. He produced the same monochloride (144) by a method of Müller (121) in which an excess of chlorine was avoided and the reaction catalyzed by a small amount of iodine.

Schorlemmer (149) also chlorinated a heptane, "ethyl amyl,"

prepared by the action of sodium on a mixture of ethyl and amyl iodides which boiled at 90–91° (accepted b. p. of heptane is 98.4°) and had a specific gravity of 0.6819 at 17.5°. (These constants are closer to those of 2-methyl- or 3-methyl-hexane than to those of *n*-heptane.) In diffused daylight chlorine was absorbed by this hydrocarbon without apparent change. The liquid then suddenly became hot, and the chlorine reacted vigorously with the evolution of hydrogen chloride. A monochloroheptane boiling at 150°, "identical with chloride of heptyl" which he had described previously (143), and a dichloropentane boiling at about 190° were found in the products. If a little iodine was added, the action continued even in the dark, with production of more highly chlorinated products. In direct sunlight the reaction took place with great vigor and separation of carbon occurred.

Schorlemmer (149) observed that heptane from petroleum gave a monochloride which boiled at 149° and had a specific gravity of 0.8965 at 19°. The monochloro derivative of heptane prepared by heating azelaic acid with barium hydroxide boiled at 151–153° and had a specific gravity of 0.8737 at 18°.

Schorlemmer and Thorpe (161) found that the product obtained by passing chlorine into the vapors of boiling *n*-heptane from *Pinus sabiniana* boiled between 143° and 157°, and consisted of 1-chloro- and 2-chloro-heptane.

Pelouze and Cahours (124) found that chlorine attacked warm heptane from American petroleum, boiling at 92–94°, with formation of a monochloroheptane which boiled at 148–152° and had a specific gravity of 0.890 at 20°.

Heptane which Morgan (118) isolated from petroleum (boiling at 96–99°) yielded a mixture of monochlorides boiling at 144–156°.

From Pennsylvania straight-run gasoline Faragher and Garner (47) separated a *n*-heptane fraction boiling at 97–100°, which they chlorinated in the liquid phase at a temperature of 20° to 25° until an increase in weight of 50 to 75 per cent of that which would be produced by complete conversion into monochloroheptane was realized. Three fractions of the product produced a monochloroheptane fraction which boiled at 150–160° at

atmospheric pressure and a dichloroheptane fraction boiling at 90–100° at 15 mm.

According to a patent of Perkin, Weizmann, and Davies (125), a stream of chlorine is passed into *n*-heptane at 90° exposed to ultra-violet rays. When chlorine was added to 300 grams of heptane until the weight of the reaction mixture had increased 50 grams, 225 grams of monochloroheptane and 10 grams of dichloroheptane were obtained. The chlorination reaction could be accelerated by heat, light, or such catalysts as phosphorus compounds or iodine.

2-Methylhexane (isoheptane). Schorlemmer (157) showed that a petroleum fraction contained small quantities of a heptane boiling at 90.4° which yielded a monochloro derivative boiling at about 150°.

Grimshaw (59) applied Schorlemmer's method (158) of halogenating in the vapor phase to the chlorination of heptane prepared from ethyl and isoamyl bromides. He observed that it was necessary to protect the reaction mixture from excessive illumination, as in strong light combustion of the chlorine-hydrocarbon mixture took place. Nearly the whole of the product boiled between 140° and 150° and consisted of monochloroheptanes. In addition, small quantities of more highly chlorinated products were always formed. Grimshaw concluded that 2-methylhexane was acted upon by chlorine in exactly the same manner as are the normal paraffins.

The photochemical halogenation process of the Badische Anilin und Soda Fabrik (9, 57, 73) has been applied to the chlorination of a fraction from Galician petroleum boiling at 88–92° and consisting mainly of isoheptane. The hydrocarbon liquid was vaporized at a pressure of 50 mm. of mercury, mixed with chlorine, and then passed through a vessel containing mercury lamps. When seven parts of chlorine to ten of hydrocarbon were used, the products consisted chiefly of a monochloroisoheptane, boiling at about 140°.

Probably the first record of the action of a chlorinating agent on isoheptane is that of Wurtz (194), who found that at its boiling point the hydrocarbon was acted upon slowly by phosphorus

pentachloride and that the action became vigorous in a sealed tube at 100°. Because the tube exploded no product was identified.

8. Octanes

Octanes appear to react slowly with chlorine in the cold, and much more rapidly if heated or illuminated. As with the heptanes, not much work has been done with pure octanes, but the raw material in most cases has been more or less closely-cut petroleum fractions. Usually chlorination experiments have been carried only to the monochloro derivative stage. More highly chlorinated products can readily be formed, however, and under extreme conditions may even break down the carbon chain of the hydrocarbon.

n-Octyl chloride and 2-chlorooctane are the only chlorine derivatives that have been positively identified. In many cases only the boiling point and density of chlorination products have been given, and structures are undetermined.

n-*Octane*. Riche (134) found that normal octane, prepared by distilling sebacic acid with barium oxide, was attacked slowly by chlorine and formed a viscous mixture of products. Schorlemmer (143) noted that the action of chlorine on octane, "hydride of octyl," yielded a monochlorooctane boiling at 170–172° and having a specific gravity of 0.892 at 18°, and also other products which could not be identified because they decomposed on distillation.

Pelouze and Cahours (124) found that the octane fraction from American petroleum, boiling at 116–118° (*n*-octane boils at 124.6°) and having a specific gravity of 0.725 at 15°, was attacked by chlorine slowly in the cold and more rapidly at 40–50°. When an excess of chlorine was avoided the main product consisted of a monochlorooctane boiling at 168–172° and having a specific gravity of 0.895 at 16°.

Schorlemmer (153) prepared from petroleum octane (boiling 122–125°) an octyl chloride which was a colorless liquid with the odor of oranges; it boiled at 173–176°, and by conversion into the alcohol was shown to be essentially 2-chlorooctane. Chlori-

nation of the octane formed by replacing the hydroxyl group in methylhexylcarbinol by hydrogen also yielded mainly a liquid boiling at 174–176°; it proved on conversion to the corresponding alcohols to be a mixture of the *primary* chloride with a small quantity of a *secondary* chloride thought to be 3-chlorooctane. It was not identical with the chlorooctane obtained from petroleum.

An octane which Cahours and Demarcay (28) separated from an oil produced by distilling crude fatty acids with superheated steam yielded *n*-octyl chloride boiling at 182°. Mabery and Hudson (103) chlorinated at ordinary temperature the vapor of an octane (b. p. 119.5–120°, specific gravity 0.7243) separated from Ohio petroleum by thirty-three fractional distillations. The best yield of monochlorooctanes was obtained when the quantity of chlorine absorbed was 50 per cent in excess of the amount theoretically required to form the monochloride. Even with this excess, a small amount of the octane remained unchanged. Fractionation of the chlorination product yielded a large quantity of a monochlorooctane boiling at 164–166°. These investigators also produced a monochlorooctane which distilled at 173–174° from a petroleum distillate boiling at 124–125°. A small amount of higher boiling material was produced which was apparently more highly chlorinated.

Other octanes. Wurtz (194) observed that hydrogen chloride was evolved when chlorine acted upon an octane (b. p. 106°, specific gravity 0.7057) prepared by the action of sodium on a butyl iodide. Antimony pentachloride also reacted with this octane, with the evolution of hydrogen chloride and the formation of a chlorine-containing product which was not investigated further. Butyl chloride, phosphorus trichloride, and hydrogen chloride were formed when phosphorus pentachloride was heated with the hydrocarbon for a long time. Later (195) Wurtz found that an octane (probably 4-methylheptane), boiling at 115–118° and having a specific gravity of 0.728 at 0°, yielded a monochloride which boiled at 167°.

Schorlemmer (151) chlorinated what was apparently diisobutyl, obtained by treating a mixture of isopropyl and amyl iodides with sodium and boiling between 109° and 110° (diisobutyl boils at

109.2°). He obtained a chlorooctane boiling at 165° and having a specific gravity of 0.8834 at 10.5°. Carleton-Williams (30) obtained a mixture of *primary* and *secondary* chlorides when diisobutyl, prepared by the action of sodium on isobutyl bromide, was chlorinated by Schorlemmer's method (158) of passing a current of dry chlorine into the vapor in diffused sunlight. The chlorinated product, which remained liquid at -17°, had no constant boiling point and could not be separated into single compounds even by repeated fractionation.

Hartmann (61) treated diisobutyl with dry chlorine under reflux, first in the cold and then at the temperature of the water-bath, as long as hydrogen chloride was evolved. The product was freed from hydrogen chloride and uncombined chlorine by placing it under a partial vacuum. It was then transferred to tubes, and iodine and antimony pentachloride added. A vigorous reaction ensued. When this had ceased, the tubes were sealed and heated at 50° and for four hours at 190-200°. They were then opened, the antimony pentachloride regenerated by passing in dry chlorine, and the tubes heated further, the temperature ultimately reaching 450°. The product consisted mainly of carbon tetrachloride, with small amounts of hexachloroethane and hexachlorobenzene.

Ahrens (1) reported, without mentioning the methods used, that the action of chlorine on an octane prepared from coal oil produced mono-, di-, and tri-chlorooctanes. The monochloride was a colorless oil boiling at 164-166°, and the dichloride a yellow oil boiling at 122-124° at 49 mm. pressure. The trichloride was not described.

9. Nonane

Both synthetic nonane and petroleum fractions falling within the nonane boiling range have been chlorinated, usually to mono-chlorononanes, but in no case has the structure of the product been proved.

Pelouze and Cahours (124) obtained a fraction of American petroleum, boiling at 136-138° and with a specific gravity of 0.741 at 15°, which from analysis and vapor density they judged

to be made up of nonane. Chlorine attacked this nonane when gently heated. A monochlorononane boiling at 196° and with a density of 0.899 at 16° was isolated.

Thorpe and Young (179) passed a stream of dry chlorine through purified nonane under reflux in direct sunlight. Action appeared to start at once, with darkening of the liquid and evolution of hydrogen chloride. "The chlorine on passing into the heated liquid appeared to burn, a flash of light appearing at the end of the delivery tube as each successive bubble of gas passed into the hydrocarbon." The passage of chlorine was stopped occasionally and the liquid distilled in order to prevent, as far as possible, the formation of highly chlorinated products. After each fractionation, the portion which distilled below 200° was exposed to further action of chlorine. Ultimately the greater part of the liquid boiled between 200° and 230°, about 40 per cent of the product boiling above 230°. A nonyl chloride fraction was obtained which boiled at 190–198° and had a specific gravity of 0.8962 at 14°; it was thought to be a mixture of *primary* and *secondary* monochlorononanes. A fraction which boiled at about 245° and was too small for analysis, was believed to be dichlorononane.

By treatment with chlorine at below 65° Lemoine (100) produced from nonane (which boiled at 132° under 759 mm. pressure and had a density of 0.730 at 23.8°) a monochlorononane distilling between 180° and 184° and having a density of 0.911 at 23.3°. He prepared monochloro derivatives from a number of other saturated hydrocarbons in the same way.

10. Decanes

n-Decane. As with other paraffins, most of the study of the chlorination of decane has been done on petroleum fractions, and very little on synthetic material. Two sorts of fractions have been used, one boiling at about the true boiling point of decane, the other at near the boiling point of isodecane. In work with fractions of both types, mono- and di-chloro derivatives have been reported, but in no case has the structure of a product been ascertained.

Besides chlorine, phosphorus pentachloride and antimony pentachloride have been used as agents of chlorination.

Decane boiling at 173–174°, which Mabery (101, 104) separated from Pennsylvania petroleum by fractional distillation, reacted rapidly with chlorine in sunlight even when the intensity of the light was diminished by interposing a sheet of newspaper. The product was fractionated *in vacuo*. After the fourth distillation, a small quantity of liquid was collected between 130° and 140° at 80 mm.; analysis indicated it to be monochlorodecane. A further fraction, distilling at 170–171° under 80 mm. pressure and at 235–240° at 747 mm. pressure and having a specific gravity of 1.0126, was shown to be a dichlorodecane.

Normal decane (101), obtained from Ohio petroleum and boiling at 173–174° gave a product boiling at 134–136° at 80 mm. and having the composition of monochlorodecane. It had a specific gravity of 0.8895 and distilled under atmospheric pressure with some decomposition at 205–210°. The chlorinated material also yielded a higher fraction, boiling from 170–180° at 80 mm., and under atmospheric pressure at 240–243° and having a specific gravity of 1.03, whose chlorine content was that of a dichlorodecane.

Decane (101) boiling at 173°, obtained from Canadian petroleum, similarly produced a monochloride, containing some dichloride, which boiled at 205–210°.

Schultz and Hartogh (164) found that chlorine acted so readily on normal decane, obtained from coal tar, that it was best to dilute the chlorine with carbon dioxide. From decane subjected to the action of diluted chlorine until the contents of the reaction flask had increased in weight about 25 per cent, they obtained a monochlorodecane fraction boiling at 180–190° at 720 mm. When decane was treated in the same manner until twice as much chlorine as in the first experiment had been absorbed, dichlorodecane was obtained, boiling at 245–250°.

The patent of Strauss (176), covering chlorination in the vapor phase under diminished pressure of petroleum hydrocarbons, brown coal tar oils, and the like, includes, as an example, the halogenation of paraffin hydrocarbon mixtures boiling between

173° and 238° and containing decane. The use of an amount of chlorine not in excess of that theoretically required to produce the monohalide, an average reaction temperature double the boiling point temperature, and the addition to the reaction mixture of a small amount of iodine are recommended.

2, 7-Dimethyloctane and decanes of unknown structure. When Wurtz treated synthetic diisoamyl (195, 196), or "diamyl," at its boiling point with chlorine, a product was obtained which distilled from 165° to above 200°. An analysis of the fraction passing over between 190° and 200° showed that it contained a monochlorodecane. By heating diisoamyl with phosphorus pentachloride, he obtained a dichloride boiling between 215° and 220° and possibly also a tetrachloride. He also found that antimony pentachloride attacked this decane.

From American petroleum Pelouze and Cahours (124) obtained a fraction, supposedly diisoamyl, with specific gravity 0.757 at 15°, and boiling point 160° (the boiling point of diisoamyl) with which, when warm, chlorine readily reacted. The first substitution product boiled at 200–204° and was shown by analysis to be a monochlorodecane.

Schorlemmer (145) found that when chlorine was passed into diisoamyl ("amyl"), a liquid resulted which boiled at about 200° and had the composition of monochlorodecane. He stated that this chloride was identical with that prepared by Pelouze and Cahours (124) from decane obtained from American petroleum.

By passing chlorine into the hydrocarbon exposed to diffused daylight, Schorlemmer (150) obtained from synthetic diisoamyl which boiled at 158–159° a monochlorodecane distilling at 203–205°. Decane, "hydride of decatyl," isolated from rectified American petroleum (b. p. 157–159°) yielded a monochloride which also boiled at 203–205°.

Grimshaw (60) passed dry chlorine into the vapor of boiling diisoamyl, using the methods he had employed with 2-methylhexane and had described in an earlier paper (59). In the presence of an excess of chlorine or of too much light there was more danger of decomposition and ignition of the decane vapor than during treatment of the heptane. The chlorinated product dis-

tilled entirely between 198° and 217°, giving two main fractions, one boiling at 198–202° and the other at 208–213°.

Mabery (101) chlorinated decane from Pennsylvania petroleum (boiling at 163–164°) in sunlight moderated by passing through a sheet of paper. Seventeen grams of chlorine reacted with 45 grams of the hydrocarbon. The chlorine was absorbed as fast as it was passed over the surface of the liquid, and hydrogen chloride was evolved. The reaction generated sufficient heat to maintain the temperature at about 70°. After ten distillations at 80 mm. pressure 6 grams of a monochlorodecane was collected at 125–130°. It had a specific gravity of 0.8914 at 20°, and at atmospheric pressure distilled at 197–203°. Eight fractionations of the higher chlorinated products yielded 15 cc. of an oil which was collected at 160–170° under 80 mm. pressure, had a specific gravity of 1.0187 at 20° and according to analysis was a dichlorodecane.

Similarly 46 grams of decane boiling at 163°, from Ohio petroleum (101), was exposed to the action of chlorine until the weight had increased 11 grams. After the chlorinated product had been fractionated twelve times at 80 mm., 15 cc. was collected at 130–135°; the fraction boiled at 200–208° under atmospheric pressure, and was shown to have the composition of a monochlorodecane. Twelve further distillations of the chlorinated product yielded 10 cc. boiling between 160° and 170° at 80 mm. The analysis indicated a dichlorodecane.

A distillate from Canadian petroleum (101), boiling at 160–161°, behaved toward chlorine as did the corresponding fractions from Ohio and Pennsylvania oils. At atmospheric pressure the monochlorodecane produced boiled at 200–204°. A distillate from Berea grit petroleum boiling at 162° also yielded a monochlorodecane, which boiled at 120–130° at 80 mm. pressure.

Lemoine (100) found that when decane with a boiling point of 155° and density of 0.908 at 19° was treated with chlorine at a temperature below 65°, it yielded a monochlorodecane which boiled at 201–203°.

Cloëz (34) obtained, among other hydrocarbons, "decane" boiling at 155–160° by the action of hydrochloric acid on cast iron, and found that the decane reacted with chlorine.

Hartmann (61) treated diisoamyl first with chlorine and then with antimony pentachloride in sealed tubes at 360°, and obtained about equal parts of carbon tetrachloride, hexachloroethane, and hexachlorobenzene, with a trace of perchloromesole, C_4Cl_6 (hexachlorobutadiene).

11. Undecane

When heated gently and treated with chlorine, undecane readily undergoes substitution. Monochloro and dichloro derivatives of the products have been separated, but no structures have been established.

From the fractionation of American petroleum, Pelouze and Cahours (124) reported an "undecane" boiling at 180–182° and having a specific gravity of 0.765 at 16°. Chlorine reacted with the hydrocarbon when heated gently. The product, a slightly amber-colored liquid, boiled mainly at 200–224°.

An undecane, boiling at 178–180° and having a specific gravity of 0.769, was separated by Cloëz (34) from the oil produced by the action of hydrochloric acid on manganiferous cast iron. Chlorine was said to act on this undecane and form substitution products, but these were not described.

Mabery (101) prepared chlorine derivatives of undecane obtained from Pennsylvania petroleum, and boiling at 196°, by allowing 40 grams of oil to absorb 14 grams of chlorine. Substitution began with the greatest readiness as soon as the chlorine came in contact with the hydrocarbon. After five distillations of the product, 10 cc. collected at 145–150° under 80 mm. pressure and distilled without decomposition between 225° and 230° at 747 mm. Its analysis corresponded to that of a monochloroundecane. Mabery believed that a dichloroundecane was also formed in the chlorination, since in the distillation at 80 mm. pressure, about 5 cc. collected at 190–200° and gave a percentage of chlorine only 2 per cent below the theoretical value for this compound.

Undecane (101), derived from Ohio petroleum and boiling at 196° gave, when subjected to chlorination, a halide which was obtained in small quantity and boiled at 150–155° under 80 mm. pressure.

To Mabery the high boiling point was evidence that the chlorination had gone so far that the monochloroundecane could not be separated completely from the dichloro derivative. From a fraction of Canadian petroleum boiling at 196°, Mabery (101) isolated a monochloroundecane fraction which boiled at 145–150° at 80 mm. and at 220–228° at atmospheric pressure.

Strauss's process (176) of chlorinating petroleum hydrocarbon vapors under diminished pressure was said to be applicable to hydrocarbon mixtures which boiled between 173° and 238° and contained undecane.

12. Dodecane

The work to date on dodecane, like that on other hydrocarbons near it in molecular weight, is fragmentary and indefinite, due to the use of petroleum fractions as starting material, and failure to identify individual products. Mainly the formation of mono- and di-chlorododecanes has been reported.

Pelouze and Cahours (124) separated from American petroleum a "dodecane" fraction which boiled at 196–200° and had a specific gravity of 0.778 at 20°. They chlorinated it with the aid of gentle heating, stopping the reaction before all of the hydrocarbon had been acted upon, and obtained as a large proportion of the product a monochloride boiling at 242–245°.

Mabery (101) allowed a dodecane, obtained from Pennsylvania petroleum and distilling completely at 214–216°, to react with chlorine until 95 grams of the hydrocarbon had absorbed 30 grams of the halogen. After six fractional distillations at 80 mm. pressure, 20 cc. of the product was collected at 142–153°. It distilled at 230–235° under atmospheric pressure. Analysis and molecular weight determinations showed it to be a monochlorododecane.

A fraction from Ohio petroleum (101), boiling at 212–214° and having a specific gravity of 0.7728, reacted readily with chlorine, and fractional distillation of the product at 80 mm. pressure yielded two main fractions, one collected at 150–160° and the other at 190–200°. The chlorine content of the 150–160° distillate was about 0.7 per cent lower than that of a dichlorododecane.

Strauss (176) claimed that paraffin hydrocarbon mixtures containing dodecane could be converted into the monochloro derivatives by treatment under diminished pressure at a temperature approximately double that of the boiling point of the hydrocarbon and preferably with the addition of a small amount of iodine.

Cloëz (34) found that chlorine attacked dodecane (density 0.782, b. p. 195–198°) obtained by treating manganiferous cast iron with hydrochloric acid. Brazier and Gossleth (23) found that in diffused daylight *n*-dodecane was attacked vigorously by chlorine and was converted rapidly into a viscous mass which could not be purified for analysis.

13. Tridecane

The work which has been done on tridecane is very indefinite, both as regards the purity of the starting material and the identity of the products formed, which usually had the chlorine content of monochloro derivatives. From American petroleum Pelouze and Cahours (124) isolated a "tridecane" fraction boiling at 216–218°, which they chlorinated in diffused light. The first substitution product boiled at 258–260°, and on the basis of a chlorine determination was called a monochlorotridecane.

After thirty-two fractional distillations, Mabery (102) obtained from Pennsylvania petroleum a hydrocarbon material boiling at 226°, which after purification had a specific gravity of 0.7834, and about the molecular weight of a tridecane. Chlorine was allowed to act on this hydrocarbon in screened sunlight over water, care being taken to avoid an excess of the halogen. After the product had been fractionally distilled several times under a pressure of 12 mm. a considerable portion was collected at 135–140°. This fraction, which had a specific gravity of 0.8974 at 20°, had the chlorine content of a monochlorotridecane.

Strauss's patent (176) gives as an example of his process for preparing monohalogen derivatives of higher paraffin hydrocarbons the reaction of a hydrocarbon mixture containing tridecane in the vapor phase under diminished pressure with a controlled quantity of chlorine.

14. Tetradecane

Pelouze and Cahours (124) chlorinated a fraction from American petroleum boiling between 236° and 240° (b. p. of tetradecane is 252.2°) and having a density of 0.809. Chlorine reacted readily and formed an amber-colored liquid which boiled at 280°, and by reason of its carbon and hydrogen content (a very poor criterion) was thought to be a monochlorotetradecane.

By twenty-four distillations at 50 mm. pressure, Mabery (102) obtained from Pennsylvania petroleum a fraction which boiled at 236–238° at atmospheric pressure. Chlorination and distillation of the product at 20 mm. gave a fraction boiling at 150–153° (specific gravity of 1.9185) which had a chlorine content 0.4 per cent higher than that of a monochlorotetradecane. A higher boiling fraction which was collected at 175–180° under 17 mm. and had a specific gravity of 1.032, had the chlorine content of a dichlorotetradecane. It should be borne in mind, however, that the differences in chlorine content of two homologous compounds, say of a chlorotetradecane and a chloropentadecane, is only 0.6 per cent, an amount so small that the halogen determinations frequently cited in proof of the identity of chlorine derivatives are almost valueless, particularly in view of the fact that the chloride fraction usually boiled over a considerable range and was made from an impure hydrocarbon material.

15. Pentadecane

As far as showing anything about the properties of pentadecane is concerned, the work which has been reported on material supposed to contain this hydrocarbon is worthless.

Chlorination by Pelouze and Cahours (124) of a fraction of American petroleum boiling between 255° and 260° (pentadecane boils at 270.5°) and having a specific gravity of 0.825 at 19°, gave a number of products, of which the lowest boiling fraction, the only one studied, was thought to be a monochloropentadecane.

The pentadecane fraction which Mabery (102) isolated by fractionating Pennsylvania petroleum distilled at 256–257° under 760 mm. pressure. After treatment with fuming sulfuric acid, its specific gravity was 0.7896 at 20°. Mabery did not succeed

in obtaining a monochloride, perhaps, in part, because he had only a small amount of the starting material. The chlorinated product boiled mainly at 175–180° under 50 mm. pressure. Chlorine and molecular weight determinations gave approximately the values for a dichloropentadecane.

Foote and Kremers (48) reported that "appreciable amounts of chlorination products" resulted from the action of nitrosyl chloride on pentadecane from *Kaempferia Galanga L.*

16. Hexadecane

A substance supposed to be hexadecane, which Mabery (102) separated from Pennsylvania petroleum and which boiled at 275–276° under 760 mm. pressure (the accepted b. p. of *n*-hexadecane is 287.5°), yielded what was apparently a dichloro derivative, distilling between 205° and 210° under 16 mm. pressure and having a specific gravity of 1.0314 at 20°.

17. Heptadecane

The fraction which boiled, after Mabery's (102) forty-second distillation of Pennsylvania petroleum, at 188–190° at 50 mm. pressure and at 288–289° at atmospheric pressure (accepted b.p. of heptadecane is 303°), and had a specific gravity of 0.800, was thought from its analysis and molecular weight to be heptadecane. The main portion of the product of its chlorination boiled at 175–177° under 15 mm. pressure, had a specific gravity of 0.8962 at 20°, and the chlorine content of a monochloroheptadecane.

18. Octadecane

Mabery (102) obtained from Pennsylvania petroleum a fraction which distilled, for the most part, at 300–301° and had a specific gravity of 0.8017. Molecular weight determinations and combustion analyses gave the data corresponding to octadecane (accepted b.p. 317°). The product obtained by the action of chlorine boiled at 185–190° under 15 mm. pressure, and had a specific gravity of 0.9041 at 20°, with the chlorine content of a monochlorooctadecane. By bringing chlorine into contact with octadecane mixed with an alkali metal or arsenic and phosphorus,

Polányi and von Bogdandy (132) claimed to produce chlorinated substances which by hydrolysis and oxidation could be converted into soap acids.

IV. BROMINATION

In general, bromination of the paraffins takes place with considerably more difficulty than chlorination. Moreover, the formation of polybromo derivatives of the paraffins proceeds to a smaller degree than does the formation of compounds containing several atoms of chlorine. Vigorous chlorination, at least of the lower paraffins, can replace the hydrogen atoms entirely; bromination, however, generally proceeds only until each carbon atom has a single bromine atom attached. Further bromination can be brought about only by most vigorous methods, which are likely to rupture the carbon chain.

The published work on bromination of these hydrocarbons is fragmentary and incomplete. On the gaseous paraffins almost no work has been done, nor is the data on the higher members of the series at all satisfying. Often only a single, monobromo derivative has been isolated, and no study given to higher products.

Schorlemmer (159) concluded "that by the action of bromine on normal paraffins only *secondary* bromides of the general formula $C_nH_{2n+1}CHBrCH_3$ are produced, but not a trace of *primary* bromide. In addition to the *secondary* bromides other products are formed, which on distillation either decompose completely, or are resolved into hydrogen bromide and unsaturated hydrocarbons which are probably olefins." However, some workers have reported the formation of *primary* bromo paraffins.

Mereshkowsky (110, 111) has attempted to coördinate the available results on the bromination of the paraffins.

"It is known from the author's experiments (bromination of propylene, isobutylene, tribromoisobutane, tetrabromoisobutane, and later tribromoisopentane) and previous work on this subject that specific catalysts not only influence the rate and initial direction of halogenation, but also determine the simultaneous production of different end-products, if the reaction is allowed to continue; the production of polyhalogenated isomerides may therefore be selectively catalysed.

The catalysts used are shown to form the following series in the order of decreasing production of the more symmetrical isomeride: (1) pure bromine without a catalyst; (2) metallic iron; (3) metallic aluminum or aluminum tribromide; (4) ferric bromide.

"The substitution rules of Markovnikov, Städel, and V. Meyer are combined and extended as follows:

(1) If the number of carbon atoms in the hydrocarbon is n , then, either with or without a catalyst at temperatures below 100°, $n - 1$ halogen atoms will become successively attached to carbon atoms on which substitution has not yet occurred.

(2) If $n - 1$ atoms of the hydrocarbon already carry a halogen atom, a further halogen atom will enter partly on the remaining halogen-free carbon atom, and partly on that one of the other carbon atoms which carries the greatest number of hydrogen atoms.

(3) Further substitution generally occurs on the carbon atom bearing the greatest number of hydrogen atoms.

(4) Substitution always occurs in several directions depending on the catalyst."

While these rules may be correct, sufficient experimental data are not available so that they may be considered proven. Except in the case of a few of the lower members of the series, the structure of the successive bromine derivatives of the individual paraffins has not been accurately determined.

1. Methane

Except in patents, there seems to be no report of a direct action of bromine on methane. Schroeter (163) patented a process for producing methyl bromide by passing a mixture of bromine and methane, or gas mixtures containing methane, over iron or other catalysts at a temperature above 200°. The iron catalyst could be replaced by small quantities of copper, copper oxide, copper carbonate, or copper bromide, by nickel, cobalt, or mixtures of these metals, or by compounds of other metals having more than one valence.

When a small amount of bromine was used, the product was reported to be about one-half unchanged methane and the remainder methyl bromide and hydrogen bromide; methylene bromide and bromoform were also formed if more bromine and a higher temperature was used.

The Elektrochemische Werke, Bosshard, Steinitz und Strauss (43) claimed the bromination of methane by mixing it with a current of gaseous bromine in the absence of catalysts, under atmospheric or elevated pressure at 300–450°. For example, 1 part of methane and 12 parts of bromine passed through quartz tubes at 400° yielded about 3.1 parts of methylene bromide, 3.1 parts of bromoform, and a small amount of carbon tetrabromide. It was reported that any of the four bromine derivatives could be obtained in predominating amount by varying the proportion of bromine to methane.

Merz and Weith (113) concluded from experiments on the bromination of methyl iodide that methane would be changed into carbon tetrabromide by direct bromination at 180°.

2. Ethane

An early patent by Mallet (107) claimed the production of bromine derivatives of ethane by passing a mixture of hydrocarbon and the halogen through a layer of animal charcoal or other porous contact material at a temperature between 30° and 90°.

That ethane would be converted into ethylene tetrabromide and hexabromoethane by the action of bromine in the presence of iodine at 200–250° was predicted by Merz and Weith (113) from experiments on ethyl iodide.

3. Propane

By using elevated temperatures, Snelling (173) was able to apply his patented chlorination process to the bromination of propane. The halogen and excess hydrocarbon reacted in a vessel from which the gas mixture was circulated into contact with a solvent to remove the halogenated product.

Merz and Weith (113) believed that they could forecast, from work on allyl bromide, the behavior of propane on energetic bromination. This substance at 210°, using iodine as a bromine carrier, yielded a liquid pentabromo derivative, which at still higher temperatures gave carbon tetrabromide, hexabromoethane, and tetra-bromoethylene.

4. Butanes

n-Butane. Butane treated with bromine under exposure to sunlight and gentle heat produced liquids consisting mainly of polybrominated compounds. Heating butane and bromine for several hours at 100° gave butylene dibromide and unidentified products.

Frankland (50) observed that bromine reacted with butane if both gases were exposed to direct sunlight and heated gently. Carius and Lisenko (29) heated bromine and butane several hours at 100° in a sealed bulb. They obtained a colorless liquid which had the bromine content of a butylene dibromide, and distilled without decomposition between 155° and 162°.

Butlerov (26, 27) obtained no decisive results from the action of one mole of bromine per mole of butane in daylight, but as a product obtained a heavy, oily, difficultly volatile material which consisted mainly of polybrominated substances.

Merz and Weith (113) continued their conjectures regarding hydrocarbon bromination by stating that because butyl bromide gave tetrabromoethylene by the action of bromine at 160–250° the same results would necessarily follow from butane. They believed that bromination would be easier than chlorination of this hydrocarbon.

Isobutane. Butlerov (26, 27) obtained no definite products from the reaction of equimolecular amounts of isobutane and bromine. As with *n*-butane, a heavy, high-boiling liquid was obtained consisting mostly of highly brominated derivatives. The bromine reacted more readily with the isobutane than with the normal hydrocarbon. Merz and Weith (113) secured from isobutyl bromide at 175° a hexabromoisobutane and at 300–340° hexabromoisobutylene. They thought they would have obtained the same results from isobutane.

5. Pentanes

Only one report of bromination of pentane has been published. Poni (133) found bromopentanes in the products of bromination of a fraction of Colibasi petroleum boiling at 0–10°. The hydrocarbon material and bromine were in contact from four to fourteen

days in the presence of water and diffused sunlight. The bromopentanes identified and their properties were as shown in table 3.

As has been mentioned, Dow (41) suggested chlorination followed by distillation to separate pentane from carbon disulfide. He also proposed bromination in the presence of iron for this purpose.

A number of workers have reported that bromine has no action on pentane; among them are Pelouze and Cahours (124), Lachowicz (87) and Routala (140).

6. Hexanes

n-Hexane. While more work has been done on the bromination of hexane than of its lower homologs, the results are sketchy and

TABLE 3
*Bromination of Colibasi petroleum fraction boiling between 0° and 10°
(Poni)*

PRODUCT	BOILING POINT
2-Bromo-2-methylbutane.....	108-109° at 745 mm. with decomposition
1-Bromo-2,2-dimethylpropane.....	89-91° at 749 mm.
1,1-Dibromo-2,2-dimethylpropane ^a	64-65° at 43 mm.
1,3-Dibromo-2,2-dimethylpropane.....	82-83° at 49 mm.

far from satisfactory. Apparently bromine reacts slowly, if at all, in the cold without light, but in sunlight, at higher temperatures, or in the presence of catalysts, reaction is quite rapid. Monobromohexanes have been isolated in pure form; dibromo derivatives also form readily, while under extreme conditions oxidation seems to take place with the formation of derivatives of benzene.

In 1860, Riche (134) observed that bromine attacked briskly a substance supposed to be *n*-hexane (b.p. 58°, specific gravity 0.688 at 0°; accepted b.p. of *n*-hexane 69°) obtained by the action of barium oxide on *n*-heptoic acid, and substitution products were

^a The constitution of the last two substances was deduced from the rule that the lower boiling of two disubstituted derivatives has the two substituting atoms closer together (Henry).

formed. Pelouze and Cahours (124) found that bromine reacted with liquid hexane and produced dibromohexane, but no monobromide could be isolated even when not more than an equimolecular quantity of bromine was used. The amount of bromine used reacted with half of the hydrocarbon present forming the dibromide, which boiled between 210° and 212°.

Wanklyn and Erlenmeyer (190) found that bromine acted with great difficulty on a hexane which boiled at 68.5–70°. When six volumes were sealed with one volume of bromine and exposed to bright sunlight for many hours, no reaction took place, nor was any effected by heating to 120°, although a change took place slowly when the mixture was exposed to sunlight after heating. Wanklyn and Erlenmeyer did not describe the products of the bromination but simply stated that "torrents of hydrobromic acid escaped" when the tube was opened.

Merz and Weith (112) assumed that hexane would brominate readily—they believed more readily than it would chlorinate—because hexyl iodide brominated smoothly at 120–130° to hexabromohexane, octabromohexane, octabromohexylene, and a compound C_6Br_8 .

In order to avoid the formation of polybromohexanes, Schorlemmer (159) allowed bromine to vaporize into gaseous hexane in the sunlight, and stopped the action when half of the hydrocarbon had reacted. The chief product was proved by conversion to the corresponding alcohol, which on oxidation with chromic acid gave acetic and butyric acid, to be 2-bromohexane.

Michael and Garner (114), using Schorlemmer's method, dropped bromine slowly into the upper part of a reflux condenser while hexane was kept boiling in a flask below. In sunlight, reaction took place instantly. The main product, boiling at 143–146°, consisted of 2-bromohexane and 3-bromohexane together with a small amount of higher boiling oil. These workers had previously found that heating hexane and bromine in sealed tubes produced polybromohexanes in considerable quantities.

Herzfelder (62) brominated *n*-hexane heated on a water bath under reflux in the presence of a spiral of iron wire. Hexabromohexane was produced when bromine and hexane were used in the

molar proportions of 7:1. Herzfelder assumed that one bromine atom combined with each carbon.

Blair, Ledbury and Wheeler (19) found that when *n*-hexane vapor was passed into bromine containing a little water, p-dibromobenzene, and *trans*-benzene hexabromide formed. The mechanism of the reaction was not clear, but since benzene volatilized into bromine by means of nitrogen yielded results identical with those given by hexane, these investigators thought it possible that, due to oxidation of the hexane by bromine, cyclohexane and then benzene were formed, and the benzene was then converted by the excess of bromine into the hexabromide and the dibromo compound.

Kaufmann and Hansen-Schmidt (77) found that 0.1 *N* solutions of bromine in carbon tetrachloride, carbon disulfide, and methyl-alcohol containing sodium bromide, left in contact with hexane in the dark remained unchanged for two weeks. When the same systems were exposed to diffused daylight for 22 days, 28 per cent of the bromine in the carbon tetrachloride solution combined with the hexane while but 3 per cent of the bromine disappeared from each of the other two solutions.

Lachowicz (87) reported that hexane withstood the action of bromine in the cold.

2,3-Dimethylbutane (diisopropyl). Silva (165) observed that if bromine and diisopropyl were permitted to react at a "mild" temperature and in sunlight a crystalline dibromo derivative was formed, but no monobromide was isolated.

7. *Heptanes*

Bromination of heptane is more satisfactory than that of any of the lower paraffins. Reaction with bromine does not occur in the dark, even in the presence of iodine or aluminum bromide, but it is rapid at water-bath temperature or in sunlight. The first product is apparently 2-bromoheptane. More highly brominated products form, but are difficult to identify because of their instability.

n-Heptane. Pelouze and Cahours (124) noted that heptane from American petroleum (b.p. 92–94°; accepted b.p. of *n*-heptane

98.4°) was not acted upon by bromine at ordinary temperature and pressure.

Schorlemmer (144) observed that hydrogen bromide was evolved slowly when a mixture of bromine and heptane, "hydride of heptyl," was exposed to direct sunlight or heated in sealed tubes in the water-bath. The action, much less energetic than that with chlorine, was hastened by the addition of a little iodine. On distillation the products decomposed with evolution of hydrogen bromide and separation of a tarry mass in the flask.

Schorlemmer (159) found that heptane was attacked slowly in artificial light, but more readily than was hexane. A bromo derivative was obtained which distilled at 165–167° and was found to be 2-bromoheptane. Much of the products decomposed during distillation into carbonaceous matter, hydrogen bromide, and heptene.

The work of Venable (186) showed that bromine did not act appreciably in the cold on normal heptane from *Pinus sabiniana* even in the presence of iodine or aluminum bromide. On the other hand, hydrogen bromide was evolved copiously when bromine was dropped into the gently boiling hydrocarbon. Best results were obtained by the addition of 75 per cent of the theoretical quantity of bromine required for the monobromide. The product was separated by distillation into three fractions: unchanged heptane; a main fraction of heptyl bromide, equivalent to about 33 per cent of the heptane; and a high-boiling mixture of other brominated and decomposition products. The monobromide boiled under ordinary pressure at 165–167°, and appeared to be identical with Schorlemmer's 2-bromoheptane. The higher boiling products were difficult to separate, since they could not be distilled without decomposition. A very unstable fraction boiling at 182–185° was not investigated. If the fraction boiling above 170° was distilled after being allowed to stand for some time, it evolved hydrogen bromide and yielded a "fair proportion" of a liquid boiling at 164–167°, which was similar to heptyl bromide.

Later, Venable (187) found when the theoretical amount of bromine for a monobromohexane was added as rapidly as it could be absorbed by hot heptane, the yield of *secondary* bromide was about as before, from 25 to 30 per cent of the theoretical.

Wheeler (191) made several unsuccessful attempts to increase Venable's yield of *secondary* heptyl bromide. His method was the same except that during the bromination he imposed an increase in pressure on the boiling heptane equivalent to 190 mm. of mercury. He also used more bromine than Venable, and by this means increased the yield of *secondary* bromide by 18 per cent, obtaining at the same time a "much greater increase" in the bromides of higher boiling point.

Francis and Young (49) treated a fraction from American petroleum, boiling between 96.5° and 102°, with bromine in the presence of aluminum bromide. Polybromo derivatives of both paraffin and polymethylene hydrocarbons were formed, but no monobromides could be isolated from the product. Without the catalyst, a petroleum fraction boiling from 93.5° to 102° was heated with bromine, and yielded a substance reported as *n*-heptyl bromide.

Cohen and Dakin (35) found that in the presence of an aluminum-mercury couple, bromine acted rapidly on a heptane fraction from light petroleum boiling at 95–100°. On fractionation of the product, a liquid boiling at 110–120° under 100 mm. pressure distilled over, which they stated was "probably heptylic bromide." The residue in the flask, on cooling, formed a colorless crystalline mass with a strong camphor-like odor. From this, two crystalline substances were obtained. The first, recrystallized from alcohol, melted at 88–90° and from its bromine content appeared to be tetrabromoheptane. The second, only slightly soluble in alcohol, crystallized from glacial acetic acid in colorless needles melting at 250°. As it contained the same percentage of bromine as the first product, it was thought that it might be polymeric with the low-melting substance. The possible structure of such a polymer was not explained.

Isoheptane. Francis and Young (49) produced an isoheptyl bromide, boiling at 83–84° under 70 mm. pressure, by heating bromine with a petroleum fraction boiling between 93.5° and 102°, and rich in isoheptane. That it was an isoheptyl derivative was proved by its reduction to isoheptane by the zinc-copper couple and hydrochloric acid.

8. Octane

Riche (134) found that bromine slowly attacked octane, prepared by distilling sebacic acid with barium oxide, and produced a viscous mixture of substitution products. On the other hand, Pelouze and Cahours (124) stated that bromine had no effect on an octane fraction from American petroleum. The octane of Pelouze and Cahours boiled between 116° and 118° (accepted b.p. of *n*-octane 124.6°) and had a density of 0.725 at 15°.

9. Decanes

n-Decane. Lachowicz (87) found that under the influence of direct sunlight, the action of bromine on normal decane was very brisk and hydrogen bromide was evolved copiously. When decane was heated to 55° with bromine it ignited and burned with a luminous flame. Berthelot (17) observed that bromine did not act on the decane obtained by the hydrogenation of "diamylene."

2,7-Dimethyloctane (diisoamyl). Lachowicz (87) reported that bromine acted very slowly on diisoamyl in diffused light, but vigorously in direct sunlight. Bromine vapor was carried by a stream of dry carbon dioxide into a reaction vessel, equipped with a reflux condenser, where the halogen mixed with the vapors of boiling diisoamyl. No brominated substance could be isolated because the product split into decylene and hydrogen bromide on distillation.

10. Dodecane

In 1851 Brazier and Gossleth (23) stated that even in sunlight bromine had practically no action on dodecane ("caproyl"), boiling at 202° (accepted b.p. of dodecane 216°), which they obtained by electrolysis of the potassium salt of *n*-heptoic acid. A supposed dodecane, boiling at 196–200° and having a specific gravity of 0.778 at 20°, which Pelouze and Cahours (124) obtained by the fractionation of American petroleum, was also unattacked by bromine in the cold. Later, Cloëz (34) found that a "dodecane" which boiled at 195–198° reacted with bromine, but he did not describe the conditions of the experiment or the products obtained.

11. Tridecane

The "tridecane" which Pelouze and Cahours (124) separated from American petroleum had a specific gravity of 0.796 at 20° and boiled at 216–218° (accepted b.p. of tridecane 234°). This "tridecane" did not react with bromine. Barbier (10) also stated that tridecane was not attacked in the cold. On the other hand, Cloëz (34) found that while an aqueous solution of bromine had no effect on this hydrocarbon, bromine itself would react with liberation of hydrogen bromide.

12. Pentadecane

Berthelot (17) reported that pentadecane did not react with bromine. Later, van Romburgh (185) found that the pentadecane which he isolated from the essential oil of *Kaempferia galanga L.* was unattacked by a solution of bromine in chloroform. Willstätter, Mayer, and Huni (193) observed that bromine did not attack a pentadecane which was produced in small amount from phytol.

V. IODINATION

It is generally stated that iodine will not react with the paraffins. This failure to substitute is attributed by Richter (135) to the reversibility of the reaction, the hydrogen iodide formed serving to reduce the alkyl iodides. This hypothesis is borne out by the fact that Datta and Chatterjee (39) were able to obtain a small amount of iodohexane by heating hexane under reflux with iodine and concentrated nitric acid, which no doubt destroyed the hydriodic acid. A "good deal" of iodohexane was hydrolyzed by the nitric acid as soon as formed. Richter (135) also states that in the presence of other substances capable of decomposing or uniting with hydriodic acid, such as iodic acid or mercuric oxide, substitution by iodine may be brought about.

VI. SUMMARY

Table 4 is a résumé of the products which have resulted from the action of chlorine and bromine upon the paraffin hydrocarbons.

TABLE 4
Substances identified in the products of the action of chlorine and of bromine on paraffin hydrocarbons

HYDROCARBON	PRODUCT REPORTED FROM ACTION OF		
	Chlorine	Bromine	
Methane	Synthetic and from natural gas	Methyl chloride, dichloromethane, chloroform, and carbon tetrachloride	Methyl bromide, dibromomethane, bromoform, and carbon tetrabromide
Ethane	Synthetic	Ethyl chloride and dichloroethane	Hexabromoethane and tetrabromoethylene
	Coal gas and natural gas	Ethyl chloride, di-, tri-, and hexa-chloroethane	
Propane	Synthetic	<i>n</i> -Propyl chloride, 1,2-dichloropropane, a trichloropropane, a tetrachloropropane (m.p. 177-178°), and hexachloropropane. Also carbon tetrachloride and hexa-chloroethane from action of chlorine and heat	
Butane	Synthetic	Butyl chloride, butylene dichloride, and "higher" products	Butylene dibromide, b.p. 155-162°
	American petroleum	Butyl chloride	
2-Methylpropane (isobutane)	Synthetic	Carbon tetrachloride and hexachloroethane	"Highly brominated derivatives"
	Pennsylvania petroleum	Isobutyl chloride and a dichlorobutane boiling at 121-122°	

TABLE 4—Continued

HYDROCARBON	PRODUCT REPORTED FROM ACTION OF		
		Chlorine	Bromine
Pentane	Synthetic	$C_5H_8Cl_4$ boiling at 230– 240°	
	American petroleum	A monochloropentane	
	Natural gasoline	Amyl chlorides, di- and tri-chloropentanes	
	Galician petroleum	<i>Primary</i> and <i>secondary</i> monochloropentanes	
2-Methylbut- tane (isopen- tane)	Galician petroleum	Monochloro derivatives	
	Ohio and Penn- sylvania pe- troleum. “Mineral” Oil	An isoamyl chloride and a dichloropentane By dry chlorination 1-, 2-, 3-, and 4-chloro- 2-methylbutane By chlorination in pres- ence of moisture— <i>primary</i> isoamyl chloride	
	Colibasi petroleum		2-Bromo-2- methylbutane
			1-Bromo-; 1,1- and 1,3-di- bromo-2,2- dimethyl- propane
2,2-Dimethyl- propane (tetra- methyl- methane)	Colibasi petroleum		
Hexane	Synthetic	1-, 2-, and 3-chloro- hexane	2- and 3-bromo- hexanes, hexa- bromohexane, <i>p</i> -dibromo- benzene and benzene hexa- bromide
	American petroleum	Mono-, di-, tri-, tetra-, and hexa-chlorohexane	Dibromohexane, b.p. 210–212°
2,3-Dimethyl- butane (diisopropyl)	Synthetic	Mono- and di-chloro- diisopropyl	Dibromodiiso- propyl
	Baku petroleum	<i>Primary</i> and <i>secondary</i> monochlorodi- isopropyl	

TABLE 4—Continued

HYDROCARBON		PRODUCT REPORTED FROM ACTION OF	
		Chlorine	Bromine
Heptane	Synthetic	A monochloride	2-Bromoheptane, b.p. 165-167°
	<i>Pinus sabiniana</i>	1- and 2-Chloroheptane	Monobromide, b.p. 165-167°
	Petroleum	A monochloride and small amounts of higher chlorides	
	Light petroleum		Mono- and tetra- bromoheptane
2-Methylhex- ane (isohep- tane)	Synthetic	A mono- and a di- chloroisooctane	
	Petroleum		Isoheptyl brom- ide, b.p. 83-84° at 70 mm.
Octane	Synthetic (dis- tillation of se- bactic acid)	Mixture of chloro- octanes	Viscous mixture of products
	Petroleum	2-Chlorooctane	
2,5-Dimethyl- hexane (di- isobutyl)	Synthetic	Mixture of <i>primary</i> and <i>secondary</i> diisobutyl chlorides	
	Synthetic (treated with antimony pen- tachloride)	Carbon tetrachloride, hexachloroethane, and hexachloro- benzene	
Nonane	American petroleum	A monochlorononane	
Decane	Synthetic		Vigorous action in sunlight, but product was not identified
	Pennsylvania, Ohio, and Canadian petroleums	A mono- and a di-chloro- decane	

TABLE 4—Continued

HYDROCARBON	PRODUCT REPORTED FROM ACTION OF		
	Chlorine	Bromine	
2,7-Dimethyl-octane (di-isoamyl)	Synthetic Synthetic (treated with antimony pen- tachloride)	A monochlorodecane Carbon tetrachloride, hexachloroethane, hexachlorobenzene, and perchloromesole (C ₄ Cl ₈)	Slow reaction in diffused light, vigorous action in sunlight
	American petroleum	A monochlorodecane	
	Pennsylvania and Ohio pe- troleums	A mono- and a di- chlorodecane	
Undecane	Pennsylvania, Ohio, and Canadian petroleums	A mono- and a di- chloroundecane	
Dodecane	Synthetic (elec- trolysis of <i>n</i> - heptoic acid) American petroleum Pennsylvania petroleum Ohio petroleum	Viscous substitution products A monochloride, b.p. 242-245° A monochloride, b.p. 230-235° A mono- and a di- chlorododecane	Only slight sub- stituting ac- tion observed
Tridecane	American petroleum Pennsylvania petroleum	A monochlorotride- cane, b.p. 258-260° A monochlorotridecane, b.p. 135-140° at 12 mm.	Did not react
Tetradecane	American petroleum Pennsylvania petroleum	A monochlorotetra- cane A monochlorotetra- cane, b.p. 150-153° at 20 mm. A dichloro- tetradecane, b.p. 175- 180° at 17 mm.	

TABLE 4—*Concluded*

HYDROCARBON	PRODUCT REPORTED FROM ACTION OF	
	Chlorine	Bromine
Pentadecane	Oil of <i>Kaempferia galanga L.</i> and also phytol	No action produced
	American petroleum	A monochloropentadecane
	Pennsylvania petroleum	A dichloropentadecane, b.p. 175–180° at 50 mm.
Hexadecane	Pennsylvania petroleum	A dichlorohexadecane, b.p. 205–210° at 16 mm.
Heptadecane	Pennsylvania petroleum	A monochloroheptadecane, b.p. 175–177° at 16 mm.
Octadecane	Pennsylvania petroleum	A monochlorooctadecane, b.p. 185–190° at 15 mm.

VII. SUGGESTED PROBLEMS FOR FURTHER RESEARCH

As a field for further study, the halogenation of the paraffins has great possibilities, and results of great interest and value can be predicted with certainty. Work is particularly needed on the liquid members of the series, using substances of known purity and accurately locating the halogen atoms in the products.

Fluorination has scarcely been attempted; chlorination has been studied considerably but not exhaustively; bromination has been investigated only in a most cursory manner; iodination is reported in but one case. All the halogens offer opportunities for fruitful research.

The following are some problems which are suggested as being worthy of consideration:

1. Chlorination in the liquid phase at low temperature, in the presence of light and with or without catalysts, in an endeavor to secure better control of the reaction.

2. Chlorination by bubbling the hydrocarbon gas or vapor through a chlorine solution or through liquid chlorine.
3. Extension of the work with chemical chlorinating agents such as antimony pentachloride, phosphorus pentachloride, sulfonyl chloride, phosgene, phosgene-aluminum chloride complexes, etc.
4. Similar investigation of the action on paraffin hydrocarbons of antimony pentabromide, phosphorus pentabromide, sulfonyl bromide, and carbonyl bromide.
5. Determination of the mechanism by which halogens convert paraffins, such as hexane, into aromatic compounds.
6. Separation of hydrocarbons by selective chlorination, possibly with the aid of catalysts.
7. Investigation of the effect of chain branching on chlorination or bromination, and determination of the reactivity of *primary*, *secondary*, and *tertiary* hydrogens toward halogens.
8. To determine whether catalysts change the point of attack of a halogen.
9. Measuring the relative rates of reaction of chlorine and bromine.
10. A study with particular thoroughness of the halogenation of hydrocarbons easily available—methane, ethane, propane, butane, and pentane.
11. Determination of the extent to which iodine substitution products can be obtained by the action of iodine on the paraffins at elevated temperatures and in the presence of substances decomposing hydrogen iodide.
12. To determine whether, as has been stated, the reactivity of paraffin hydrocarbons to bromine decreases on ascending the series.
13. Further work on chlorination with carriers or promoters for the purpose, in part, of discovering new aids to reaction.
14. Further work on the action of active chlorine on paraffin hydrocarbons.
15. Determination of the relative ease of chlorination of individual paraffin hydrocarbons and their lower chlorine derivatives.

16. A study of the effect of the chloro derivatives first formed on the further course and extent of the halogenation.

17. Observation of the effect of halogenation in the presence of aqua regia and of mixtures of nitric and hydrobromic acids on paraffin hydrocarbons.

18. Determination of the action of bromine vapor on vapors of boiling, individual, paraffin hydrocarbons with and without solid finely-divided or porous catalytic contact materials.

19. Halogenation in the presence of silent discharge under low temperature conditions.

REFERENCES

- (1) AHRENS: Ber. 40, 848-52 (1907).
- (2) ASCHAN: Ber. 31, 1802 (1898).
- (3) ASCHAN: Översikt Finska Vetenskaps-Soc. Förh. 58, 122 (1915); Chem. Zentr. 1918, II, 939-41; J. Chem. Soc. 118, i, 277-80 (1920); C. A. 14, 3654 (1920).
- (4) AYRES: Canadian pat. 278,537, March 13, 1928; U. S. pat. 1,717,136, June 11, 1929; U. S. pat. 1,741,393, Dec. 31, 1929; Ind. Eng. Chem. 21, 899-904 (1929). Compare THE B. A. S. Co.: French pat. 657,518, July 16, 1928; C. A. 23, 4228 (1929).
- (5) BADISCHE ANILIN UND SODA FABRIK: British pat. 5,125, Feb. 29, 1912; conv. date Oct. 30, 1911.
- (6) BADISCHE ANILIN UND SODA FABRIK: French pat. 435,312, Oct. 14, 1911; J. Soc. Chem. Ind. 31, 409 (1912).
- (7) BADISCHE ANILIN UND SODA FABRIK: German pat. 261,677, April 14, 1911.
- (8) BADISCHE ANILIN UND SODA FABRIK: German pat. 263,716, Oct. 31, 1911, addition to 261,677.
- (9) BADISCHE ANILIN UND SODA FABRIK: German pat. 266,119, Oct. 31, 1911.
- (10) BARBIER: Ann. chim. phys. [5] 7, 510 (1876).
- (11) BASKERVILLE AND HAMOR: J. Ind. Eng. Chem. 4, 216 (1912).
- (12) BASKERVILLE AND RIEDERER: J. Ind. Eng. Chem. 5, 5-8 (1913).
- (13) BAUER: Compt. rend. 51, 572-3 (1860).
- (14) BEDFORD: J. Ind. Eng. Chem. 8, 1090-4 (1916).
- (15) BEDFORD: U. S. pat. 1,245,553, Nov. 6, 1917.
- (16) BERTHELOT: Compt. rend. 45, 916-20 (1857); Ann. chim. phys. [3] 52, 97-103 (1858); Ann. 105, 241-5 (1858).
- (17) BERTHELOT: Bull. soc. chim. [2] 11, 15-30 (1869).
- (18) BISCHOFF: Edinburgh New Phil. J. 30, 152 (1840-41).
- (19) BLAIR, LEDBURY AND WHEELER: J. Soc. Chem. Ind. 43, 289-90T (1924).
- (20) BLANC: U. S. pat. 1,248,065, Nov. 27, 1917.
- (21) BOSWELL AND McLAUGHLIN: Canadian Journal of Research 1, 240-55 (1929).
- (22) BOYD: U. S. pat. 1,293,012, Feb. 4, 1919.
- (23) BRAZIER AND GOSSLETH: J. Chem. Soc. 3, 228 (1851).
- (24) BROOKS, ESSEX AND SMITH: U. S. pat. 1,191,916, July 18, 1916.

- (25) BROOKS, SMITH AND ESSEX: *J. Ind. Eng. Chem.* **10**, 511-5 (1918).
- (26) BUTLEROV: *Ann.* **144**, 15-7 (1867).
- (27) BUTLEROV: *Comm. Univ. Kasan*, 1867; *Z. für Chem.* **10**, 363 (1867).
- (28) CAHOURS AND DEMARCAVY: *Compt. rend.* **80**, 1568-72 (1875).
- (29) CARIUS AND LISENKO: *Ann.* **126**, 214-7 (1863).
- (30) CARLETON-WILLIAMS: *J. Chem. Soc.* **31**, 542 (1877); **35**, 126 (1879).
- (31) CARTER AND COXE: U. S. pat. 1,572,513, Feb. 9, 1926; Canadian pat. 251,763, July 14, 1925. Compare WADE (Karpen and Bros.): British pat. 245,991, April 27, 1925; KARPEN AND BROS.: German pat. 472,421, Feb. 28, 1929.
- (32) CHEMISCHE FABRIK AUF AKTIEN: Austrian pat. 63,819; *J. Chem. Soc.* **106**, i, 917 (1914).
- (33) CLARK: *Ind. Eng. Chem.* **22**, 439 (1930).
- (34) CLOEZ: *Compt. rend.* **85**, 1004 (1877).
- (35) COHEN AND DAKIN: *J. Chem. Soc.* **75**, 896 (1899).
- (36) COLIN: U. S. pat. 427,744, May 13, 1890.
- (37) CURME: U. S. pat. 1,422,838, July 18, 1922.
- (38) DARLING: *Ann.* **150**, 217-23 (1869).
- (39) DATTA AND CHATTERJEE: *J. Am. Chem. Soc.* **39**, 439 (1917).
- (40) DOBEYANSKII AND GUREVICH: *Neftyanoe Khozyaistvo* **15**, 532-59 (1928); *C. A.* **23**, 4811 (1929).
- (41) DOW: U. S. pat. 1,317,359, Sept. 30, 1919.
- (42) DUMAS: *Ann. chim. phys.* [2] **73**, 94-5 (1840); *Ann.* **33**, 187-91 (1840).
- (43) ELEKTROCHEM. WERKE, BOSSHARD, STEINITZ, UND STRAUSS: German pat. 391,745, Jan. 25, 1921; *J. Soc. Chem. Ind.* **43**, B811 (1924).
- (44) ELEKTROCHEM. WERKE, BOSSHARD, STEINITZ, UND STRAUSS: German pat. 413,724, July 7, 1921.
- (45) ELWORTHY: *Ind. Chemist* **4**, 275-8 (1928).
- (46) ELWORTHY AND LANCE: French pat. 354,291, May 15, 1905; *J. Soc. Chem. Ind.* **24**, 1083 (1905).
- (47) FARAGHER AND GARNER: *J. Am. Chem. Soc.* **43**, 1717-24 (1921).
- (48) FOOTE AND KREMERS: *Am. J. Pharm.* **100**, 600 (1928).
- (49) FRANCIS AND YOUNG: *J. Chem. Soc.* **73**, 920 (1898).
- (50) FRANKLAND: *Ann.* **71**, 202,205 (1849).
- (51) FRANKLAND: *J. Chem. Soc.* **3**, 338-41 (1851).
- (52) GARLICK: *Ind. Chemist* **4**, 363-7 (1928).
- (53) GARNER: *Nat. Gas and Gasoline J.* **12**, 328-9 (1918); *Gas Age* **41**, 559 (1918); *Am. Gas. Eng. J.* **108**, 491,506 (1918); *Proc. Nat. Gas. Assoc.* **10**, 136 (1918).
- (54) GARNER AND CLAYTON: U. S. pat. 1,262,769, April 16, 1918.
- (55) GAULT AND BENLIAN: *Chimie & industrie*, Special No., p. 272 (May, 1924); *C. A.* **18**, 3109 (1924).
- (56) GOLDSCHMIDT A.-G.: British pat. 227,475, conv. date (Germany) Jan. 10, 1924. Compare WEBER AND ERASMUS: U. S. pat. 1,565,345, Dec. 15, 1926.
- (57) GRAUL AND HANSCHKE (Assrs. to Badische Anilin und Soda Fabrik): U. S. pat. 1,032,822, July 16, 1912.
- (58) GRAUL, HANSCHKE AND WEBEL: U. S. pat. 1,298,929, April 1, 1919.
- (59) GRIMSHAW: *J. Chem. Soc.* **26**, 312, 318 (1873); *Ann.* **166**, 163 (1873).

- (60) GRIMSHAW: J. Chem. Soc. **32**, 261-2 (1877); Ber. **10**, 1602 (1877).
- (61) HARTMANN: Ber. **24**, 1011-26 (1891).
- (62) HERZFELDER: Ber. **26**, 2432-8 (1893); J. Chem. Soc. **66**, 1 (1894).
- (63) HOCHSTETTER: German pat. 292,089, Nov. 20, 1914.
- (64) HOLZVERKOHLUNGS-IND. A.-G. AND ROKA: British pat. 186,270, Nov. 22, 1921.
- (65) HOLZVERKOHLUNGS-IND. A.-G.: British pat. 195,345, conv. date (Germany) March 27, 1922.
- (66) HOLZVERKOHLUNGS-IND. A.-G.: German pat. 378, 137, Oct. 19, 1920.
- (67) HOLZVERKOHLUNGS-IND. A.-G.: German pat. 393,550, Sept., 1921.
- (68) HOLZVERKOHLUNGS-IND. A.-G.: German pat. 477,494, Dec. 21, 1921; C. A. **23**, 3982 (1929).
- (69) HOLZVERKOHLUNGS-IND. A. G.: German pat. 478,083, Nov. 16, 1923; C. A. **23**, 4228 (1929).
- (70) HUFF: Trans. Am. Electrochem. Soc. **36**, 180 (1919).
- (71) I. G. FARBEININDUSTRIE A.-G.: German pat. 486,952, Jan. 16, 1930.
- (72) INTERNATIONAL FIREPROOF PRODUCTS CORP.: Brazilian appl. 6,051; French pat. 650,738, March 10, 1928; C. A. **23**, 3341 (1929).
- (73) JOHNSON (Badische Anilin und Soda Fabrik): British pat. 4,766, Feb. 26, 1912.
- (74) JOHNSON (Badische Anilin und Soda Fabrik): British pat. 18,356, Aug. 14, 1911.
- (75) JONES AND ALLISON: J. Ind. Eng. Chem. **11**, 639-43 (1919).
- (76) JONES, ALLISON AND MEIGHAN: Bur. Mines Tech. Papers **255**, 44 pp. (1921).
- (77) KAUFMANN AND HANSEN-SCHMIDT: Arch. Pharm. **263**, 32-50 (1925).
- (78) KIRKPATRICK: Chem. Met. Eng. **34**, 276-8 (1927).
- (79) KOCH: U. S. pat. 1,432,761, Oct. 24, 1924.
- (80) KOCH AND BURRELL: Ind. Eng. Chem. **19**, 442-5 (1927).
- (81) KOCH AND STALLKAMP: U. S. pat. 1,380,067, May 31, 1921.
- (82) KOLBE: Ann. **76**, 37 (1850).
- (83) KOLBE AND FRANKLAND: J. Chem. Soc. **1**, 65-9 (1849).
- (84) KRAFFT AND MERZ: Ber. **8**, 1045, 1296-1302 (1875).
- (85) KRAUSE (to Holzverkohlungs-Ind. A.-G.): U. S. pat. 1,677,831, July 17, 1928.
- (86) KRAUSE AND ROKA: U. S. pat. 1,654,821, Jan. 3, 1928.
- (87) LACHOWICZ: Ann. **220**, 173-92 (1883).
- (88) LACY: U. S. pat. 1,111,842, Sept. 29, 1914; U. S. Re. 14,105, April 11, 1916; British pat. 16,194, July 7, 1914.
- (89) LACY: U. S. pat. 1,190,659, July 11, 1916.
- (90) LACY: U. S. pat. 1,242,208, Oct. 9, 1917.
- (91) LACY: U. S. pat. 1,245,742, Nov. 6, 1917.
- (92) LACY: U. S. pat. 1,263,906, April 23, 1918.
- (93) LACY: U. S. pat. 1,286,353, Dec. 3, 1918; British pat. 101,708, conv. date Oct. 6, 1915; Canadian pat. 202,994, Aug. 17, 1920; Canadian pat. 203,250, Aug. 24, 1920.
- (94) LACY: U. S. pat. 1,308,760, July 8, 1919.
- (95) LACY: Canadian pat. 173,969, Dec. 19, 1916.
- (96) LACY: French pat. 480,064, June 15, 1916; C. A. **11**, 1276 (1917).
- (97) LACY: French pat. 483,093, May 25, 1917; C. A. **12**, 42 (1918).

- (98) LAMB, WILSON AND CHANEY: Ind. Eng. Chem. **11**, 421 (1919).
- (99) LEISER AND ZIFFER: U. S. pat. 1,459,777, June 26, 1923.
- (100) LEMOINE: Bull. soc. chim. [1] **41**, 163-5 (1884).
- (101) MABERY: Proc. Am. Acad. Arts Sci. **32**, 121-76 (1897); Am. Chem. J. **19**, 419-82 (1897).
- (102) MABERY: Proc. Am. Acad. Arts Sci. **37**, 565-95 (1902); Am. Chem. J. **28**, 165-98 (1902).
- (103) MABERY AND HUDSON: Proc. Am. Acad. Arts Sci. **32**, 101-18; Am. Chem. J. **19**, 243-62 (1897).
- (104) MABERY AND HUDSON: Proc. Am. Acad. Arts Sci. **32**, 179-82 (1897); Am. Chem. J. **19**, 482-5 (1897).
- (105) MACKAYE: U. S. pat. 880,900, March 3, 1908.
- (106) MACKAYE: U. S. pat. 1,009,428, Nov. 21, 1911.
- (107) MALLET: U. S. pat. 220,397, Oct. 7, 1879.
- (108) MARTIN AND FUCHS: Z. Elektrochem. **27**, 150-62 (1921).
- (109) MELSENS: Ann. chim. phys. [2] **74**, 110 (1840); J. prakt. Chem. **21**, 266 (1840).
- (110) MERESHKOWSKY: Ann. **431**, 113-32 (1923); J. Chem. Soc. **124**, i, 526 (1923).
- (111) MERESHKOWSKY: Bull. soc. chim. [4] **37**, 864 (1925).
- (112) MERZ AND WEITH: Ber. **10**, 402, 1234 (1877); **11**, 2247 (1878).
- (113) MERZ AND WEITH: Ber. **11**, 2235-53 (1878).
- (114) MICHAEL AND GARNER: Ber. **34**, 4036-40 (1901).
- (115) MICHAEL AND TURNER: Ber. **39**, 2153-6 (1906).
- (116) MOISSAN: Compt. rend. **110**, 952 (1890).
- (117) MOISSAN AND CHAVANNE: Compt. rend. **140**, 409 (1905).
- (118) MORGAN: Chem. News **30**, 292 (1874); J. Chem. Soc. **28**, 301-6 (1875); Ann. **177**, 304-11 (1875).
- (119) MOTT AND BEDFORD: J. Ind. Eng. Chem. **8**, 1033 (1916).
- (120) MÜLLER, E.: Z. Elektrochem. **31**, 49 (1925).
- (121) MÜLLER, H.: J. Chem. Soc. **15**, 41 (1862); Ann. chim. phys. [3] **46**, 199 (1863).
- (122) PAYNE AND MONTGOMERY: U. S. pat. 1,453,766, May 1, 1923.
- (123) PAYNE AND MONTGOMERY: U. S. pat. 1,520,506, Dec. 23, 1925; Canadian pat. 268,301, Feb. 8, 1927.
- (124) PELOUZE AND CAHOURS: Compt. rend. **54**, 1241-7 (1862); **56**, 505-13 (1863); **57**, 62-9 (1863); Ann. chim. phys. [4] **1**, 1-93 (1864); J. prakt. Chem. **88**, 314-6; **89**, 359-61 (1863); **91**, 98-100 (1864).
- (125) PERKIN, WEIZMANN AND DAVIES: British pat. 22,653, Oct. 4, 1912.
- (126) PFEIFER AND MAUTHNER: British pat. 157,253, conv. date (Hungary) Nov. 20, 1918.
- (127) PFEIFER, MAUTHNER AND REITLINGER: J. prakt. Chem. **99**, 239-42 (1919).
- (128) PFEIFER AND SZARVASY: German pat. 242,570, April 21, 1910; U. S. pat. 1,012,149, Dec. 19, 1911.
- (129) PHILLIPS: Trans. Am. Phil. Soc. **17**, III, 181 (1893); Am. Chem. J. **16**, 263 (1894).
- (130) PHILLIPS: Trans. Am. Phil. Soc. **17**, III, 210-2 (1893); Am. Chem. J. **16**, 361-3 (1894).
- (131) POLÁNYI AND VON BOGDANDY: French pat. 653,163, April 20, 1928; C. A. **23**, 3713 (1929).

- (132) POLÁNYI AND VON BOGDANDY: British pat. 289,795, April 29, 1927; C. A. 23, 847 (1929). Compare POLÁNYI: Trans. Faraday Soc. 24, 606 (1928).
- (133) PONTI: Ann. Sci. Univ. Jassy 3, 212-6 (1905); J. Chem. Soc. 90, 1 (1906); Chem. Zentr. 1906, I, 442.
- (134) RICHE: Compt. rend. 50, 815-7 (1860); Ann. 117, 266-8 (1861).
- (135) RICHTER: Organic Chemistry, translation by Spielmann, Vol. I, p. 92. Blakiston's Son and Co., Philadelphia (1919).
- (136) RIESENFELD: U. S. pat. 1,455,508, May 15, 1923.
- (137) ROKA (Assr. to Holzverkohlungs-Ind. A.-G.): U. S. pat. 1,723,442, Aug. 6, 1929.
- (138) RÖMER: Dissertation, Tübingen (1885); Ann. 233, 186, 196-8 (1886).
- (139) RONALDS: Trans. Roy. Soc. Edinburgh 23, 496 (1864); J. Chem. Soc. 18, 60 (1865).
- (140) ROUTALA: Ann. acad. sci. Fennicae [A] 2, No. 13, 19 pp.; Chem. Zentr. 1912, II, 638.
- (141) SCHLEEDE AND LUCKOW: Ber. 55B, 3710-26 (1922).
- (142) SCHLEGEL: Ann. 226, 133-74 (1884).
- (143) SCHORLEMMER: J. Chem. Soc. 15, 423-5 (1862); Ann. 125, 109-12 (1863).
- (144) SCHORLEMMER: J. Chem. Soc. 16, 217 (1863); Ann. 127, 314 (1863); Bull. soc. chim. [2] 1, 188 (1864).
- (145) SCHORLEMMER: J. Chem. Soc. 16, 425-9 (1863); J. prakt. Chem. 92, 193-9 (1864); Ann. 129, 243-9 (1864); Ann. chim. phys. [4] 1, 493-4 (1864).
- (146) SCHORLEMMER: Proc. Roy. Soc. (London) 13, 225-7 (1864); Compt. rend. 58, 703-4; (1864); Ann. chim. phys. [4] 3, 456-7 (1864); Ann. 131, 76-9 (1864).
- (147) SCHORLEMMER: J. Chem. Soc. 17, 262-5 (1864); Ann. 132, 234-8 (1864).
- (148) SCHORLEMMER: Proc. Roy. Soc. (London) 14, 167 (1865).
- (149) SCHORLEMMER: Proc. Roy. Soc. (London) 14, 169, 466, 468 (1865); Ann. 136, 259, 266 (1865); Z. für Chem. 8, 244 (1865).
- (150) SCHORLEMMER: Proc. Roy. Soc. (London) 14, 173 (1865); Z. für Chem. 8, 247 (1865).
- (151) SCHORLEMMER: Proc. Roy. Soc. (London) 16, 35-8 (1867); Ann. 144, 186-90 (1867); Z. für Chem. 10, 1, 75 (1867).
- (152) SCHORLEMMER: Proc. Roy. Soc. (London) 17, 372-6 (1868); Ann. 150, 210-4 (1869); Ann. chim. phys. [4] 19, 439-41 (1870).
- (153) SCHORLEMMER: Proc. Roy. Soc. (London) 18, 25-32 (1869-70); Ann. 152, 153-63 (1869).
- (154) SCHORLEMMER: Ber. 3, 615-6 (1870); Phil. Trans. 162, 112 (1872).
- (155) SCHORLEMMER: Proc. Roy. Soc. (London) 19, 20-2 (1871).
- (156) SCHORLEMMER: Phil. Trans. 162, 114 (1872); Ann. 161, 268-9 (1872).
- (157) SCHORLEMMER: Phil. Trans. 162, 120-1 (1872); Ann. 161, 278-9 (1872).
- (158) SCHORLEMMER: Phil. Trans. 162, 112-3, 116, 118-9 (1872); Ann. 161, 265-7, 272, 275 (1872).
- (159) SCHORLEMMER: Phil. Trans. 169, 50-2 (1879); Proc. Roy. Soc. (London) 26, 325-6 (1878); Ann. 188, 250-6 (1877); Bull. soc. chim. [2] 30, 189-90 (1878).
- (160) SCHORLEMMER: Proc. Roy. Soc. (London) 29, 364-5 (1881); Ann. 199, 139-44 (1879); Chem. News 40, 280 (1879); Phil. Trans. 171, 451-4 (1881).

- (161) SCHORLEMMER AND THORPE: Proc. Roy. Soc. (London) **34**, 405 (1886); Phil. Trans. **174**, 269-71 (1884); Ann. **217**, 149-52 (1883); Am. Chem. J. **6**, 28-31 (1884).
- (162) SCHÖYEN: Ann. **130**, 233-7 (1864).
- (163) SCHROETER: German pat. 330,642, Nov. 25, 1917.
- (164) SCHULTZ AND HARTOGH: Ber. **42**, 3611 (1909).
- (165) SILVA: Ber. **5**, 984 (1872); **6**, 36 (1873).
- (166) SILVA: Ber. **7**, 953 (1874).
- (167) SNELLING: U. S. pat. 1,271,790, July 9, 1918.
- (168) SNELLING: U. S. pat. 1,285,823, Nov. 26, 1918.
- (169) SNELLING: U. S. pat. 1,325,214, Dec. 16, 1919.
- (170) SNELLING: U. S. pat. 1,339,675, May 11, 1920.
- (171) SNELLING: U. S. pat. 1,365,740, Jan. 18, 1921.
- (172) SNELLING: U. S. pat. 1,420,346, June 20, 1922.
- (173) SNELLING: U. S. pat. 1,421,733, July 4, 1922.
- (174) SPARRE: U. S. pat. 1,148,258, July 27, 1915.
- (175) SPER: U. S. pat. 1,721,873, July 23, 1929.
- (176) STRAUSS: German pat. 267,204, April 24, 1912; Chem. Zentr. **1913**, II, 2013.
- (177) SZARVASY: German pat. 336,205, Feb. 3, 1918; British pat. 159,843, March 4, 1921.
- (178) TANAKA: J. Soc. Chem. Ind. (Japan) **24**, 789-98 and Abstracts 68-9 (1921); C. A. **16**, 1309 (1922).
- (179) THORPE AND YOUNG: Proc. Roy. Soc. (London) **21**, 196-7 (1873); Ann. **165**, 20-1 (1873).
- (180) TISSIER: Ann. chim. phys. [6] **29**, 344 (1893).
- (181) TIZARD, CHAPMAN AND TAYLOR: British pat. 214,293, Dec. 14, 1922.
- (182) TOLLOCZKO: Abhandl. Krakauer Akad. Wiss. **A52**, 307-16 (1912); Chem. Zentr. **1913**, II, 99-100; J. Soc. Chem. Ind. **32**, 742 (1913); C. A. **8**, 1282 (1914).
- (183) TOLLOCZKO AND KLING: Abhandl. Krakauer Akad. Wiss. **A52**, 295-306 (1912); Chem. Zentr. **1913**, II, 98-9; J. Soc. Chem. Ind. **32**, 742 (1913); C. A., **8**, 1282 (1914).
- (184) TOMPKINS: British pat. 780, Jan. 18, 1915.
- (185) VAN ROMBURGH: Verslag Akad. Wetenschappen Amsterdam **10**, 622 (1901-2); Proc. Acad. Sci. Amsterdam **4**, 619-20 (1902).
- (186) VENABLE: Ber. **13**, 1650 (1880); Dissertation, pp. 8-14, Göttingen (1881).
- (187) VENABLE: Am. Chem. J., **10**, 237-9 (1888).
- (188) VIVAS: U. S. pat. 1,733,843, Oct. 29, 1929; British pat. 286,726, conv. date March 11, 1927.
- (189) WALTER: German pat. 222,919, Nov. 5, 1909; Chem. Zentr. **1910**, II, 255.
- (190) WANKLYN AND ERLENMEYER: J. Chem. Soc. **16**, 234 (1863).
- (191) WHEELER: J. Am. Chem. Soc. **25**, 532-4 (1903).
- (192) WHISTON: J. Chem. Soc. **117**, 183-90; J. Soc. Chem. Ind. **39**, 384 (1920).
- (193) WILLSTÄTTER, MAYER AND HÜNI: Ann. **378**, 121 (1910).
- (194) WURTZ: Ann. **96**, 366-71 (1855).
- (195) WURTZ: Compt. rend. **58**, 1167-8, 1246 (1863); Ann. **128**, 231-3 (1863).
- (196) WURTZ: Bull. soc. chim. [1] **5**, 312-5 (1863).
- (197) YONEYAMA AND BAN: Japanese pat. 35,701, Jan. 29, 1920; C. A. **14**, 3428 (1920).

THE CRYSTALLIZATION, DENATURATION, AND FLOCCULATION OF PROTEINS, WITH SPECIAL REFERENCE TO ALBUMIN AND HEMOGLOBIN; TOGETHER WITH AN APPENDIX ON THE PHYSICOCHEMICAL BEHAVIOR OF GLYCINE

W. C. M. LEWIS

*The Muspratt Laboratory of Physical and Electro-Chemistry, University of Liverpool,
Liverpool, England*

Received July 12, 1930

CONTENTS

Certain aspects of the behavior of denaturable proteins, in particular egg albumin and hemoglobin, in respect to crystallization, denaturation and flocculation, are reviewed, a number of closely related problems necessarily coming under consideration at the same time.

The various topics discussed are grouped under the following heads:

I. CRYSTALLIZATION

The possible significance of the electric moment of certain groupings; the work of Miss Chick and Martin upon the composition of crystalline egg albumin precipitated by ammonium sulfate (prior to the subsequent washing process); the results of Sörensen on the composition of the finally purified egg albumin.

II. DENATURATION

1. *The general features of denaturation by heat and by acid or alkali*

The probable unimolecular character of the process; the lack of precise knowledge regarding the nature of the chemical and physical changes involved; the identity in titratability (by acid) of denatured and undenatured egg albumin; the reopening of the question of the reversibility of denaturation by the results of Anson and Mirsky.

2. *The extent of interaction of undenatured protein with acid and alkali*

The evidence that over a considerable pH range (from the iso-electric point outwards) protein salts (e.g., albumin chloride (or sulfate) and sodium albuminate) are practically completely ionized, but, that beyond certain pH limits appreciable quantities of unionized salt exist in solution; the chemical union versus adsorption problem; the hydrolysis of albumin chloride and sodium albuminate, and the average net charge on the protein ions at different pH values.

*3. Behavior of denatured protein with respect to acid and alkali**4. The question of the extent of union of undenatured and denatured protein with neutral salts*

The work of Pauli and his collaborators, and of Northrop and Kunitz; the conclusion that both ions of an inorganic salt simultaneously combine to a slight extent (and reversibly) with protein, but that the ions are combined in general in non-equivalent amounts; the difference in behavior of denatured and undenatured serum albumin towards silver ion (Pauli and Matula); the problem of the positions of attachment of inorganic ions with protein.

5. Alteration in pH as a consequence of union of protein with neutral salt

A method of determining the difference in extent of the union of inorganic anion and cation.

6. The solvent action of neutral salts and of non-electrolytes upon undenatured and denatured protein

The work of Hardy and of Mellanby on the globulins; the problem of peptization; the work of Cohn and Miss Prentiss on the solubility of hemoglobin in salt solution in terms of the Debye-Hückel theory of interionic attraction; the effective valency of hemoglobin and the globulins; the zwitterion hypothesis of Bjerrum and the interionic attraction theory; the possible importance of the dielectric capacity of protein solutions in regard to solubility effects; the Bjerrum-Larsson "distribution" equation; the effect of urea upon protein solubility.

III. FLOCCULATION

Observations of Aronstein, of Heynsius, and of Miss Chick and Martin; the rôle of neutral salt (in small amount) in flocculation; the polymerization of glycine; the possible distinction between true flocculation (i.e., chain formation produced by addition of successive protein units) at or near the iso-electric point, and the precipitation of protein *salt* in an amorphous form at a pH removed from the iso-electric point; contrast between the precipitating action of an acid upon a denatured protein and upon a colloidal metal, respectively.

The critical increment of flocculation: observations of Miss Chick and Martin; influence of temperature on rate of flocculation of protein and of a colloidal metal; the nature of the problem raised by the observations of Miss Chick and Martin upon the rapid fall of the critical increment with rise in temperature.

APPENDIX

The physicochemical behavior of glycine in solution.

INTRODUCTION

In the first place it is necessary to recall the definitions attaching to the three terms, crystallization, denaturation, and flocculation.

Crystallization is the precipitation in the solid crystalline form of undenatured protein. This material is obtained as a rule by

salting out with ammonium sulfate of appropriate concentration and subsequent washing. According to the careful work of Sörensen on egg albumin, referred to later, the crystalline material is best obtained not at the iso-electric point ($\text{pH} = 4.8$) but at $\text{pH} = 4.58$. It consists therefore of protein sulfate having a sulfate content of 0.43 per cent. The crystalline protein (i.e., the sulfate) is easily soluble in the pure solvent water.

Denaturation is a change of a kind not yet understood which the fresh (undenatured) protein is capable of undergoing in the presence of water. It is frequently brought about by heating the protein solution. It may likewise be brought about at ordinary temperature by addition of sufficient acid or alkali. The denaturation process is unimolecular in nature so far as the protein itself is concerned, and appears to be associated with some internal physical or chemical change generally regarded as being of an irreversible nature within the structure of each protein unit. Denaturation appears to be a definite step in the transition of the fresh undenatured (and crystallizable) protein molecules to the amorphous state represented by flocculation.

Flocculation is the precipitation of protein in the neighborhood of the iso-electric point in an amorphous form, this form not dissolving in the pure solvent (water). Flocculation is possible only provided the original protein has suffered the change known as denaturation. The two consecutive changes—namely, denaturation plus flocculation—taken together are frequently referred to as *coagulation*. In order that flocculation may occur with measurable speed a small amount of an electrolyte (neutral salt) must be present even at the iso-electric pH. In the total absence of such electrolyte the protein may be denatured but it will not flocculate, except on boiling and then only provided the pH be adjusted close to the iso-electric point.

The coagulum, formed as a consequence of flocculation, although insoluble in the pure solvent, will dissolve when sufficient acid or alkali is added. This, however, does not entail reversal of the denaturation step itself.

So much for the terms crystallization, denaturation and flocculation; we have now to consider in greater detail certain points in connection with the phenomena associated with these terms.

I. CRYSTALLIZATION

In the first place, the fact that crystalline undenatured protein can be obtained at all, demonstrates that mere proximity of such molecules is not in itself sufficient to bring about the coagulation change—i.e., denaturation plus flocculation. Since, however, crystallization is itself brought about in the presence of (much) salt, and since we know that even a small amount of salt is sufficient to bring about flocculation of material already denatured, it follows that neither close approximation of protein molecules nor the presence of much ammonium sulfate at ordinary temperatures is capable of bringing about denaturation. One concludes that the undenatured protein molecules are relatively inert. They are however, titratable—i.e., the acidic and basic portions are capable of interacting with an added acid or alkali without undergoing denaturation, as has been shown conclusively by Booth (1) in the light of his own data and of the data of P. S. Lewis and of Cubin. An excess of such reagent will, however, bring about denaturation even at room temperature.

To return to crystallization. The function of the (concentrated) ammonium sulfate in bringing about crystallization of undenatured protein is usually regarded as that of a dehydrating agent. The dehydration view implies that there is a greater tendency for either ion of the salt to associate itself with a water molecule dipole than with the amino or carboxyl dipoles situated on the protein molecule. This tendency will be directly proportional to the electric moment of the dipole of the water molecule or of the protein group respectively. Williams (2) has given a table for the characteristic moments of certain groups based upon the dielectric capacity of solutions (in benzene) of benzene derivatives of different types. Benzene itself is taken as non-polar. It is thus found that the electric moment, μ , for the NH_2 group is 1.5×10^{-18} e.s.u. For gaseous alkyl amines it is known that $\mu = 1.31 \times 10^{-18}$. For the carboxyl group $\mu = 0.9 \times 10^{-18}$. On the other hand, for water molecules in the gaseous state (monohydrol) the electric moment is distinctly higher, namely, 1.87×10^{-18} .

So far as these values go they are in agreement with the pre-

ferential attraction of the salt for the water molecules. It is necessary however to draw attention to the fact that μ for liquid (polymerized) water is only 0.5×10^{-18} (cf. Fowler (3)). If water were entirely composed of stable H_4O_2 molecules we would therefore expect the salt to adhere to the protein in preference to the water. It is probably right to infer that in hydration we have actually to do mainly with oriented unpolymerized H_2O molecules.

In the foregoing considerations it has been tacitly assumed that the amino and carboxyl groups of the protein molecule are in the undissociated or unionized form. Certain of these groups, however, taken in pairs, are very likely to give rise to the zwitterion structure usually represented by $^+NH_3 \cdot R \cdot COO^-$. (Actually in proteins it is more probable that the zwitterion formation arises from the mutual influence of contiguous branches thus, $R \cdot NH_3^+$ and $-OOC \cdot R$, the positively charged portion corresponding to the terminal (ϵ) amino group of a diamino unit such as lysine, or the analogous group in arginine, or histidine, while the negatively charged portion belongs to the terminal carboxyl in a dicarboxylic unit such as aspartic or glutaminic acid). Although represented in the above manner the positively and negatively charged groups do not function as free and independent ions but are so placed as to cause a partial mutual saturation or neutralization of their electric fields. In fact, if this mutual interference did not occur, the zwitterion formation would be scarcely likely to persist, union with the H^+ ion and OH^- ion in the solvent transforming the zwitterion to the "classical" form. So far as attractive forces are concerned, therefore, the type and extent of interaction between salt, water, and protein would not be expected to be much affected whether we assume an undissociated structure or a zwitterion structure.

In view of the fact that the dipole moments of an amino group, a carboxyl group, and a water molecule, although not the same in magnitude are at least of the same order of magnitude, it would be expected that the ions of a salt such as ammonium salt, while exhibiting preferential affinity for the water molecule, would also attach themselves to a certain extent to the appropriate groups of the protein. It would be anticipated therefore,

that the precipitate obtained by salting out a protein such as egg albumin by means of ammonium sulfate would contain (prior to further washing and purification) not only protein but salt and water as well. This is shown by the classical work of Chick and Martin (4), who determined the composition of the solution in equilibrium with the precipitate under various conditions, as well as the composition of the precipitate freed as far as possible from adhering mother liquor by pressure, the pressure employed in this case being 3 tons per square inch. In the table of results given by these authors the percentage of protein in the pressed precipitate varies from 64 per cent to 73 per cent. At the same time the $(\text{NH}_4)_2\text{SO}_4$ varies from 30 per cent to 22 per cent, and the water from 8.5 per cent to 6.4 per cent. (These are rounded values given simply by way of illustration.) It is evident that the amount of water remaining in the pressed precipitate is determined by the inorganic salt present therein. This points to the water being associated principally with the salt, in agreement with the Hofmeister concept of the dehydrating action exerted by the salt upon the protein prior to precipitate formation.

It is of interest to look at the matter briefly from the point of view of relative dipole moments. The problem is essentially the distribution of the ions of the salt between the water dipoles and protein dipoles present simultaneously. The probability of attachment between salt ion and water dipole would be expected to be given by $(1 - e^{-E_{iw}/RT})$ where E_{iw} is the energy required to break the ion-water union. A similar expression involving E_{ip} would be expected for the ion-protein complex. Since the two energy terms are of the same order of magnitude we would expect to find appreciable quantities of both ion-water and ion-protein complexes in the system, as in fact we do find. It is to be observed that the condition contemplated in the salting-out process is that in which there is a high concentration of salt, that is, the condition is favorable to *partial* as distinct from complete hydration of the salt. The ions of the inorganic salt present possess consequently a considerable stray field wherewith to compete for further water dipoles and for protein dipoles. On increasing the active mass of the water (as by washing), the condi-

tion becomes favorable for the preferential union of salt with water rather than of salt with protein, so that the protein is obtained salt-free. It would be interesting to know what sort of effects would be produced by the addition of, say, a non-electrolyte such as sugar which would further compete for the water dipoles.

In the case of flocculation of iso-electric protein on the other hand, the electrolyte required is present usually in small concentration. There is abundant water present to saturate the ions of the salt, with the result that although the ions function locally to favor aggregation of the denatured protein units (compare the section on flocculation), the conditions are not favorable for permanent chemical union between protein and salt ions. The protein aggregates are therefore formed practically free from the precipitating salt. If this view be correct it follows that the degree of hydration of the ions of the salt plays an important part. Thus it is implied that if excess salt be used in connection with flocculation, union of salt and protein should now be possible, not because there is much salt but because of the partial dehydration of the ions. This would conceivably result, not in flocculation, but in peptization (compare section on solvent action of neutral salts); or, alternatively, if flocculation occur, it would be expected that the salt should form a definite part of the material thrown out of solution. Experimental evidence of this nature seems to be lacking.

To return to crystallization. Since the function of the ammonium sulfate is taken to be that of a dehydrating agent, the question arises as to whether its place can be taken by another salt with strong affinity for water. So far as present experience goes (cf. Sörensen (5) and Sörensen and Palitzsch (6)), sulfates stand out as by far the best precipitating agents, although their place may be taken by phosphate, arsenate, and citrate. These authors themselves leave the matter in some doubt. Further information is obviously required in this connection. As regards alcohol, Preyer (7) uses this in small quantities in his method of obtaining crystalline hemoglobin. If this material be in fact undenatured crystalline protein it would indicate that ions of

salts such as $(\text{NH}_4)_2\text{SO}_4$, even in small quantities, do not necessarily constitute a characteristic feature of the crystalline state. It has to be borne in mind, however, that alcohol in appreciable amount brings about denaturation of protein.

The most careful experimental investigation on the production of pure protein by crystallization is that of Sörensen (*loc. cit.*) on crystalline egg albumin, using ammonium sulfate as the precipitating salt.

As already mentioned, Sörensen finds that precipitation occurs best at pH = 4.58. The crystalline material was washed several times with sodium chloride solution until it was impossible to detect any sulfate in the filtrate. The material was finally treated with water, the greater part of it dissolving and some of it, apparently denatured, remaining undissolved. Sörensen remarks that "the results of our experiments must be interpreted as showing that it is not possible from such washing experiments to draw any conclusions as to whether the crystals contain ammonium sulfate or not." In the light of the results obtained by Sörensen using his "proportionality method," it becomes almost certain, however, that ammonium sulfate as such is not a necessary constituent of the crystalline protein material finally obtained. At the pH employed, however, the crystalline material contains the sulfate radical. Sörensen expresses this by saying that there is approximately 1 equivalent of sulfuric acid to 125 equivalents of (total) protein nitrogen. Booth has calculated from this, on taking the most recent and apparently most reliable value for the molecular weight of (undenatured) egg albumin, namely 43,000 (Marrack and Hewitt (8)), and also making use of the fact that the mass of nitrogen in egg albumin is 15.51 per cent of the total mass (Mathews (9)), that at the pH = 4.58 there are four equivalents of sulfate combined with one molecule of albumin. Thus the egg albumin sulfate obtained on precipitation has the formula, albumin - $(\text{SO}_4)_2$.¹

¹ To attempt to ascribe a stoichiometric formula to a protein salt even in the "solid" state is naturally a doubtful procedure in view of the tendency of such material to produce micelles (i.e., aggregates of numerous molecules with occluded solvent and ions) when dissolved. It may, however, be pointed out that such

A point arises here. At the above pH, when this precipitate is dissolved the ionization may be regarded as complete. That is, in solution we will have albumin in the form in which its molecule carries four positive charges. As we shall see later at a much lower pH range the albumin sulfate is far from being completely ionized. This suggests that the albumin cation would conceivably react with the OH⁻ of the solvent to give some "undissociated" base which would in fact be iso-electric undenatured albumin itself. If this occurs it would not be permissible to regard the crystalline albumin sulfate of Sörensen as a single substance; it should be regarded as a mass of mixed crystals of sulfate and iso-electric crystalline albumin. As will be shown later, however, the pH range over which hydrolysis in the above sense is appreciable is so minute that even at pH = 4.58 it is already virtually absent. On the whole, it is probably safe to regard the washed crystalline material obtained at pH = 4.58 as a single entity.

II. DENATURATION

1. *General considerations*

The first question which naturally arises here is whether denaturation is a general phenomenon in the transition of a protein molecule or unit from the fresh natural dissolved state to the flocculated state. In this connection it is to be remembered that simple denaturation is a process which can be brought about with relative ease by heating or at ordinary temperatures, by the action of chemical reagents such as acid or alkali in sufficient amount, or by alcohol and probably by other organic solvents.

evidence as exists, e.g., the magnitude of viscosity, suggests that while micelle formation is the outstanding feature of certain proteins, notably gelatin, in the dissolved state, molecules as such, but in the hydrated form, appear to predominate in the case of egg albumin solutions. It is probable that this is true in particular for solutions of fresh undenatured egg albumin. After denaturation has taken place and the pH adjusted within certain limits, the conditions for incipient flocculation thus produced may lead likewise to micelle formation even for this protein.

Consequently, if the actual preparation or isolation of a given protein involves such conditions or reagents the resulting material, if it had originally been capable of denaturation, would be likely to have become denatured prior to any systematic investigation of its physicochemical behavior. In such cases it is impossible to say whether the intramolecular change denoted by denaturation has occurred at any stage in the history of the protein or whether such change is incapable of occurring, i.e., whether the phenomenon of denaturation is non-existent in certain proteins. In other words, it is impossible to state with certainty whether denaturation is a general feature of protein reactivity or not. Among the simple proteins which are "heat coagulable" and therefore presumably denaturable by heat are the albumins, globulins and possibly glutelin. In the group of conjugated proteins hemoglobin stands out as definitely denaturable. It is very doubtful, however, whether casein (caseinogen) is denaturable; probably it is not. Incidentally, the change of casein to paracasein by means of rennet has been shown by Palmer and Richardson (10) to involve marked increase in acid and alkali binding capacities on the part of the protein over the entire range of pH examined, and we may consequently infer that this change is of a much more profound character than would be demanded by denaturation alone. Probably no protein has been so intensively examined from a physicochemical point of view as gelatin. From its mode of preparation, however, which involves relatively drastic hydrolytic conditions, it may be concluded with certainty that gelatin is already denatured or more exactly, for this protein, denaturation may be regarded as having no meaning. Further, aqueous "solutions" of gelatin are apparently much more complex than are solutions of albumin. Of the proteins the methods of preparation of which do not bring about denaturation, crystalline hemoglobin and crystalline egg albumin are the most prominent examples. For this reason, in discussing denaturation and the properties of solutions of undenatured and denatured protein we are forced to confine ourselves very largely to these two proteins.

The unimolecular velocity constants of denaturation in the case of these two proteins have been determined by Chick and

Martin (11), and later in the writer's laboratory by P. S. Lewis (12), Cubin (13) and Booth (unpublished work) over a wide range of pH and at different temperatures. It has been shown for both egg albumin and hemoglobin that the velocity of denaturation is a minimum not at the iso-electric point of the protein but at the neutrality point of water. The iso-electric point has no special significance whatever for the act of heat denaturation.

The fact that denaturation is a strictly unimolecular process in respect to the protein affords *prima facie* evidence that the process is not one of aggregation of protein units. A review of the literature on osmotic pressure measurements tends to confirm this, although at the present time no systematic work upon protein deliberately denatured has been carried out, by either the osmometer or centrifuge method. It is evidently desirable that such measurements be carried out by someone familiar with specialized technique of this kind.

As already mentioned, the precise nature of the chemical change, if any, which occurs in denaturation is quite unknown. The fact that the process is catalyzed to approximately the same extent by H^+ ion and OH^- ion, but that at the same time no change in the H^+ ion concentration accompanies it, serves to eliminate a number of plausible chemical changes. Thus the absence of change in the H^+ ion concentration and the identity in titratability of denatured and undenatured proteins (Booth (1)) rule out the possibility of associating denaturation with hydrolysis of a peptide linkage, unless indeed there be a simultaneous and *exactly equivalent* change in the opposite sense elsewhere in the molecule.^{1a} Even if hydrolysis of some kind were shown to occur it would not in itself be particularly helpful, unless it could be shown that this change was accompanied by a change in configuration rendering the contiguous amino and carboxyl groups more easily available for the subsequent flocculation process, for which denaturation is regarded as a favorable preliminary step.

It is to be remembered that the observed critical increment or energy of activation in denaturation is excessively great—of the

^{1a}That denaturation may involve hydrolysis of a peptide link and simultaneously union of amino and carboxyl elsewhere was suggested to the writer by Professor Ramsden.

order of 100,000 calories per mole in the neighborhood of the neutrality point. An energy term of this magnitude suggests as the most significant alteration in the protein unit an actual physical distension or opening up of the structure, possibly accompanied by the breaking of bridge-like linkages in the original undenatured unit. The act of denaturation on the part of a single protein molecule requires the presence of an appreciable amount of water. This is shown by the well-known experiments of Chick and Martin (*loc. cit.*) in which proteins "freed as much as possible from moisture by squeezing in a press between filter papers, but still containing 20 per cent of water" could be heated in a current of air at 120°C. for five hours without becoming denatured. Denaturation obviously is not brought about by a small amount of water, but appears to require water in bulk or at least amounts of water vapor such as are produced from a heated water bath (cf. Chick and Martin, *loc. cit.*).

The suggestion that denaturation may be accompanied by physical distension of the protein molecule in solution is likewise in agreement with the increase in viscosity attributed to the change (Chick (14)). Further, the fact that denatured protein is more easily attacked than is undenatured protein by proteolytic enzymes² in the act of digestion points to a more penetrable and less tightly bound structure. Also the denaturing effect ascribed by Chick to lecithin may be associated with this substance's well known peptizing capacity, an effect also likely to open up a molecular structure. The fact, however, that globulins are brought into solution, i.e., peptized by neutral salts and that nevertheless the resulting peptized globulin, e.g. edestin, is stated to be denaturable, indicates that denaturation and pepti-

² Talarico (15) has shown this fairly conclusively for the action of trypsin on egg albumin. His experiments likewise indicate that when coagulation has been carried far by prolonged heating the resulting material, as one would expect, becomes less digestible. Lin, Wu and Chen (16) also find that the denatured material is on the whole the more digestible, although protein denatured by alcohol is more digestible by trypsin but less digestible by pepsin than is undenatured protein. Linossier (17) finds that egg albumin denatured by heat is less digestible by pepsin than is the unheated material. In general apparently no great effort has been made to allow for the interfering effect of flocculation.

zation *per se* are not necessarily closely related phenomena. This is emphasized by the fact that while iodide ion and sulfo-cyanide ion are good peptizing agencies they both markedly raise the "temperature of denaturation," i.e., they inhibit denaturation (Pauli and Handovsky). It does not appear to be quite certain, however, how far the effect of these ions is exerted upon the denaturation process as distinct from the subsequent flocculation process.

The influence of salts upon the rate of denaturation is considerable; the mechanism however remains obscure. Lepeschkin (18) has investigated the phenomenon fairly extensively. As an example of the marked accelerating effect of salts of the "heavy" metals, one may cite the behavior of Ba^{++} . Using egg albumin (Kahlbaum) dialyzed for 6 days, Lepeschkin observed that "in presence of 0.1 N KCl the average time of denaturation was found at 75°C. to be 2095 seconds, while in the presence of 0.1 N $BaCl_2$ denaturation proceeded instantaneously at the same temperature; in the presence of 0.003 N $BaCl_2$ it required 95 seconds, 0.003 N $MgCl_2$ 120 seconds, 0.0001 N HCl 52 seconds, while it proceeded instantaneously in the presence of 0.003 N HCl." Lepeschkin points out that the effect of salts is so marked as "to make necessary the suggestion that the salts in question first enter into chemical unions with proteins which are soluble in water and react with it more easily than the free proteins." While union is highly probable, there is as yet no clear reason why the denaturation is thereby facilitated. That the influence of salt upon the rate of denaturation is of a dual nature is shown by the results of P. S. Lewis, who found in certain cases that the velocity passed through a maximum with increase in salt content. The same dual effect has been observed by Pauli and Handovsky in connection with the socalled "temperature of heat denaturation," a mode of examining denaturation which has been superseded by the method of velocities and velocity constants. The phenomenon of the influence of salts upon denaturation on the whole has not been systematically examined with adequate pH control. Little can therefore be said about it at the present time.

Owing to the great variation in the rate of denaturation with

hydrogen ion and hydroxyl ion concentration, it is impracticable to work over a wide pH range at a single temperature. Measurements are made at different (suitable) temperatures, and with the aid of the critical increments (which are themselves a function of pH and become enormously great as we approach neutrality from either side) it is possible to calculate at least approximately what the velocity constants would be at a given single temperature over a wide pH range. In table 1 the temperature chosen is 25° and as a comparative measure of the rate of denaturation the

TABLE 1
Time required for 50 per cent denaturation of proteins at : °C.

pH	Days	Hours	Minutes
Crystalline egg albumin			
1.02		12	6
1.35		23	30
1.99	3	20	—
2.55	9	3	—
3.83	139	—	—
5.13 to 8.29	Time varies between 560 and 29600 years		
Crystalline hemoglobin (from ox blood)			
4.08	—	—	6.5
4.41	—	—	31
4.77	—	3	46
5.10	—	17	6
5.50	4	14	—
7.0	291	—	—

times for half denaturation have been computed. These results are of practical value as giving some idea of the time that may safely be spent in dialyzing etc. without danger of appreciable denaturation. It is evident that the rate of denaturation of egg albumin at ordinary temperatures is exceedingly slow compared with that of hemoglobin. The enormous critical increment of denaturation in the pH region in the neighborhood of neutrality is the cause of the almost unbelievably slow rate given in table 1.

As there is apparently some difference of opinion regarding the

feature or features which characterize and accompany denaturation it is necessary to state here the sense in which the term is employed by the present writer. It may be put simply thus. A protein dissolved in water may be said to be undenatured if no flocculation occurs when the pH is adjusted to the iso-electric point of the protein and if at the same time a small amount of neutral salt be present in the system. Denaturation is most conveniently brought about by heating in the presence of water. It may also be brought about by addition of sufficient acid or alkali, but in such cases it is essential that excess of the reagent be avoided in order that no unnecessary chemical changes be brought about at the same time. The statement not infrequently made that denaturation is accompanied by production of acid, i.e., by definite hydrolysis, is undoubtedly true, but such changes are unnecessarily violent and are not essential to the denaturation process itself.

As already mentioned denaturation is regarded as irreversible, the process going to completion under all ordinary circumstances. The whole question of the reversibility of denaturation has recently been raised, however, chiefly by the striking results obtained by Anson and Mirsky (19), who believe that they have obtained fairly conclusive evidence that denaturation may be reversed in the case of hemoglobin and hence, by inference, for other proteins. The method of obtaining soluble undenatured hemoglobin from coagulated hemoglobin is as follows. "If an acid or alkaline solution of completely coagulated hemoglobin is neutralized all the protein is precipitated. If, however, before complete neutralization the solution of denatured hemoglobin is allowed to stand in slightly alkaline solution under conditions to be described, then, on complete neutralization only a part of the protein is precipitated. The remaining soluble part is the apparently reversed hemoglobin. How much of this soluble, apparently reversed hemoglobin is obtained depends, other factors being constant, on the state of the prosthetic group, heme, and on the species of the hemoglobin."

The features of denaturation and the question of its reversibility bear some analogy to the hydrolysis of sucrose. Under all

ordinary conditions the hydrolysis of sucrose by acid to glucose and fructose is complete. There is no reliable evidence of combination of the resultants, not because of absence of collision, for which the conditions are favorable, but because of the fact that one of the hexose moieties (the fructose portion) as it exists in the intact sucrose molecule is in a five-membered ring form.³ On hydrolysis this form changes almost entirely to a six-membered ring form, and interaction between this and the glucose cannot produce sucrose though it might well produce another disaccharide. Of course in the case of denaturation the "resultants" are not separated from the parent molecular structure, but, by the setting up of a new configuration within the original structure involving new spatial arrangements of atomic groups and distribution of valency forces, the probability of reproducing all the initial conditions simultaneously is so small as to be negligible. On this basis, for all practical purposes denaturation would be irreversible; it does not eliminate the possibility of reversal however. The surprising thing is that a change in environmental conditions relatively as minor as that described by Anson and Mirsky should have any appreciable influence on reversal at all. The whole problem is obviously now under revision and at the present stage it would be premature to speculate on the outcome.⁴

2. Behavior of undenatured protein with respect to acid and alkali

In connection with the mode of union of a strong acid, HCl, with egg albumin, reference may be made in the first place to experiments carried out a long time ago by Bugarsky and Liebermann (22). These authors do not state definitely whether the albumin was denatured or not, but it may be inferred from their paper that it was almost certainly undenatured to begin with, although prolonged contact with the most concentrated acid solution employed

³ For an examination of the significance of this structure in relation to the critical increment of hydrolysis of sucrose and other glucosides, cf. Moelwyn-Hughes (20).

⁴ In connection with the solvent action of certain salts (e.g., sulfocyanides and salicylates) upon coagulated albumin (cf. Willheim (21)) it is not clear whether denaturation is here reversed or simply prevented.

would probably bring about denaturation. Measurements with hydrogen ion concentration cells and chloride ion concentration cells were carried out, and their results show definitely that in the presence of HCl both H⁺ and Cl⁻ combine with the protein. The initial concentration of HCl, to which various amounts of albumin are added, was 0.05 N. With 6.4 grams albumin in 100 cc., 96.56 per cent of the original H⁺ is combined, while 76 per cent of the Cl⁻ is combined. In view of the general reactivity of H⁺ (and OH⁻) it is not surprising that the union of H⁺ with the protein should be as extensive as these data show. It is somewhat surprising, however, to find evidence of marked union of Cl⁻ to form unionized protein chloride. With the concentrations involved one would have expected the protein chloride to be very largely ionized. The protein salt, in fact, appears to be much more sensitive (in respect to ionization) to the presence of a strong electrolyte with a common ion than would be the case with sodium chloride, for example. Although the general behavior of egg albumin with respect to acid is illustrated qualitatively by the results of Bugarsky and Liebermann, the extent of union with the chloride ion thus indicated is somewhat misleading. Manabe and Matula (23) were the first to show definitely that Cl⁻ is in general less combined with the protein than is H⁺, for HCl concentrations not exceeding 0.05 N. With very dilute HCl the protein salt is practically completely ionized. Actually Manabe and Matula examined serum albumin, but there is no reason to doubt that their conclusion is applicable in principle to egg albumin as well. Using a 1 per cent solution of serum albumin in the presence of HCl originally 0.02 N, the H⁺ bound is 0.014 while the Cl⁻ bound is 0.005. The latter figure corresponds to the concentration of unionized salt, whence it may be concluded that the degree of ionization under these conditions is of the order 64 per cent. In the presence of HCl originally 0.04 N, the degree of ionization is only of the order 25 per cent.

Reference may likewise be made to electrometric measurements by Rohonyi (24), who employed Merck's presumably undenatured crystallized egg albumin, as well as other material. With a 1.9 per cent egg albumin solution in HCl, initially 0.05 N,

Rohonyi finds that the concentration of bound H⁺ ions in equivalents per liter is 0.0183, the concentration of bound Cl⁻ ions being almost the same, *viz.* 0.0170. It follows that the concentration of free H⁺ ions in equilibrium with this amount of albumin is 0.0317 N (pH = 1.5), and the apparent equivalent of albumin at this pH is 1040. The "effective" concentration of the protein as salt is 0.0183 N, and from the figures given the degree of ionization in respect to Cl is only about 7 per cent. Incidentally albumose (Witte peptone) behaves very similarly to the egg albumin, while alanine under similar conditions is ionized considerably more (43 per cent), but by no means completely. Attention may also be drawn to the results obtained by Chick and Martin (25) upon the union of undenatured crystalline egg albumin (in 0.95 – 2.0 per cent solutions) with sulfuric acid. The hydrogen electrode was employed, thus giving information regarding the union of hydrogen ion with the protein. Similar electrode measurements with the SO₄²⁻ or HSO₄⁻ ion are of course impossible,⁵ so that no information is available as regards the degree of ionization of the sulfate. These authors find a general resemblance between their results and those of Bugarsky and Leibermann, although apparently considerably greater union occurred in the case of sulfuric than in the case of hydrochloric acid. On the other hand Loeb (*Proteins and the Theory of Colloidal Behavior*, p. 49 (1922)) finds that over the pH range 4.2 – 2.4 (the entire range quoted in Loeb's table), the union of 0.1 N sulfuric and 0.1 N hydrochloric acids with 1 per cent crystalline egg albumin is the same, as one would expect on simple stoichiometric grounds. In Loeb's table just referred to, the largest amount of sulfuric acid recorded as combined with one gram of albumin happens to be 7.0×10^{-4} equivalents. (Due allowance has been made in the titration experiments for the titration capacity of the solvent in arriving at this figure.) The pH corresponding to this degree of combination is 2.4. If this were the limiting value the apparent equivalent weight would be 1430. In Chick and Martin's experiments the recorded number of equivalents of sulfuric acid combined is 1.79×10^{-3} , this occurring at a pH = 1.65.

⁵ Northrop and Kunitz (26) have suggested and employed a method for such cases as this based upon Donnan's Membrane Theory.

On this basis the apparent equivalent weight of albumin is as low as 560. The number of titratable amino groups per gram of egg albumin is 64.9×10^{-5} . On this basis the effective equivalent weight of albumin is 1540. This corresponds on the basis of Loeb's data to the titratability of egg albumin at a pH = 2.6. It was suggested long ago by Brailsford Robertson that the titratability of a protein was greater, in general considerably greater, than would be expected from the free amino groups, and for this reason he proposed his well-known concept of the titratability of the —CONH— grouping⁶ in addition to that of the free amino groups themselves. Whether the actual "additional" titration is to be ascribed to the —CONH— grouping or not, it would appear very probable that groupings of some kind would be involved in a stoichiometric sense. It would probably lead to clearness if it were generally agreed to regard the equivalent weight arbitrarily as that which corresponds to the number of free amino groups, i.e., the value would be about 1540 in the case of albumin.

As a matter of fact, the above stoichiometric view of the "additional" titratability (assuming this to be real) have been subjected to considerable criticism, particularly by those who draw a sharp distinction between mass action combination and adsorption. The distinction is supposed to be emphasized by ascribing mass action binding to primary valency, adsorption to secondary valency. Thus Gortner (cf. Hoffman and Gortner, (27)), who has investigated a considerable number of vegetable proteins as well as casein, concludes that "the amount of acid bound at acidities lying between the iso-electric point of the protein and pH 2.5 was definitely correlated with the free amino nitrogen of the protein. . . . Accordingly, in this region the acid binding was different for the various proteins and was dependent upon the chemical composition of the proteins. At greater acidities the further acid binding was independent of the

⁶ The "titratability" of the peptide grouping is here attributed to addition of acid to the *intact* grouping. There is no evidence for Robertson's idea of fission of this linkage merely by the act of titration, much more drastic conditions being required for this purpose. Cf. Escolme and Lewis (28).

chemical composition of the proteins and was related only to protein concentration. At acidities greater than pH = 2.5 all proteins bound gram for gram the same amount of acid." Presumably "all" is not to be taken quite literally. It may be pointed out, however, that even on an adsorption basis the same amount of binding need not necessarily occur.

As Hitchcock has pointed out, it is impossible to distinguish between a true mass action combination and adsorption which obeys Langmuir's equation, that is, adsorption at a certain fraction of a finite number of "places" on a surface.⁷ Incidentally, some of the criticism levelled against Loeb's conclusions is for this reason pointless. Considering in the first place the reactivity of the protein from the iso-electric point out to a pH = 2.6, we undoubtedly have a finite number of groups situated on any single protein molecule, these groups being titratable by the appropriate reagent. To regard such interaction as a chemical process, as does Loeb for example (and over this pH range Gortner is in agreement with Loeb), would seem to be a not unreasonable way of looking at the matter. If we prefer to call such interaction adsorption, we imply adsorption in the Langmuir sense. Apparently those who emphasize the view of adsorption as sharply distinguished from mass action have in mind an effect, which they ascribe to secondary valency as distinct from primary valency, in which the adsorbed molecules or ions are not located at definite positions in or on the protein molecule structure, but are distributed statistically over the "surface" of the molecule. Such an effect, if it occurs, would be likely to be minor compared with the localized and specific adsorption at definite groups; nor is such a view essential to the production of the electrokinetic p.d. (potential difference). At the present time it is probably unnecessary to appeal to such a general type of adsorption in the case of a

⁷ In connection with the union of Cl⁻ ion with dissolved glycine (aminoacetic acid) examined by Oryng and Pauli (29), these authors point out that the curve obtained by plotting extent of combination against the free chloride ion content of the system gives rise to a curve which "has the typical form for an adsorption process." As Oryng and Pauli themselves point out, the term adsorption in this connection has no physical meaning. The general shape of the curve cannot therefore be taken as a criterion.

complex molecule such as that of a protein wherein there are numerous groupings having marked specific reactivity.

Returning to the work already considered upon the union of acid with dissolved undenatured albumin to produce, in solution, protein salt of variable composition—the composition being a function of the pH—it may be pointed out that when such salt is obtained in the solid form it is probably crystalline. We have in mind a degree of acidity not sufficient of itself to bring about denaturation even at ordinary temperatures (25°C.). If, however, the concentration of acid is greater than this, i.e., greater than 0.1 *N* in the case of egg albumin and greater than 10^{-4} *N* in the case of hemoglobin, denaturation could occur. In such a case it does not seem to be known with certainty whether the salt precipitated would be crystalline or amorphous. Presumably an appeal would have to be made to the x-ray method of investigation.

Returning to the question of the degree of ionization of protein chloride in solution, reference may be made to two investigations of relatively recent date—namely, that of Hitchcock and that of Frisch, Pauli and Valko. Measurements on the interaction of hydrochloric acid (up to 0.1 *M*) with the proteins, gelatin, egg albumin, casein, edestin and serum globulin (in solutions containing one gram of protein in 100 cc.) have been carried out by Hitchcock (30) using the electromotive force method. The electrodes are the hydrogen and silver-silver chloride electrodes, respectively. The data cover a considerable range and are apparently of a high degree of accuracy. The results have been calculated simply on a concentration basis, not on an activity basis, and consequently would require recalculation to estimate with precision the degree of ionization at different pH values. The conclusion reached by Hitchcock is that the protein chlorides are highly ionized except in the most acid regions studied.

Electrometric determinations of the behavior of egg albumin and other proteins towards strong acid—employing the hydrogen and calomel electrodes respectively—have been carried out by Frisch, Pauli and Valko (31). The range of acid employed is approximately that covered by Bugarsky and Liebermann,

but the data are more numerous and presumably very much more accurate. The theoretical treatment involves activity considerations instead of the earlier concentration relations. The method of calculation is not particularly clear and the writer is unable to follow certain of the steps. It is possible, however, to make a partial use of the data presented in order to check the conclusions already drawn as regards the degree of ionization of the albumin chloride. Thus using 1 per cent albumin solution dissolved in HCl initially approximately 0.05 N (accurately 0.0492 N), the activity of the uncombined Cl⁻ expressed as a "corrected concentration" is found to be 0.0353 N. Employing the activity coefficient obtained by Pauli and Wit (32) for Cl⁻ ion in a 0.05 N HCl solution, namely $\alpha = 0.831$, it follows that the concentration of free Cl⁻ ion is $0.0353 / 0.831 = 0.0425$ N. Consequently the quantity of Cl⁻ bound to the protein in the form of unionized albumin chloride is $(0.0492 - 0.0425)$ or .0067 equivalents per liter. The authors give 0.0103 expressed in equivalents per liter as the quantity of H⁺ ion bound by protein. If the protein chloride at the pH in question (namely, 1.41 corresponding to the equilibrium (free) H⁺ ion 0.0389 N) were completely unionized, the concentration of bound Cl⁻ would likewise be 0.0103. It follows that the fraction of albumin chloride ionized is $\frac{0.0103 - 0.0067}{0.0103}$, or 35 per cent.

Again, with 1 per cent albumin in the presence of HCl of initial concentration 0.03 N (accurately 0.0295 N) the activity of the free Cl⁻ is found to be 0.0220. Taking the Pauli-Wit α value 0.850 for Cl⁻ in HCl of this concentration, it follows that the concentration of free Cl⁻ ion is 0.0259 N, whence the concentration of bound Cl⁻ is 0.0036. Since the concentration of bound H⁺ is 0.010, it follows that the degree of ionization with respect to Cl⁻ ion is $\frac{0.10 - 0.0036}{0.010}$, or 64 per cent. A similar calculation for 1 per cent albumin in 0.0197 N HCl gives a dissociation of 75 per cent, while in 0.00984 N HCl the dissociation is practically complete. That is to say, between pH = 2 and the iso-electric point, pH = 4.8, the albumin chloride (containing

1 gram protein in 100 cc.) may be regarded as completely ionized.⁸

As regards union of undenatured albumin with alkali, the usual titration experiments indicate the capacity of the protein to combine with hydroxyl ion. This is borne out also by the e.m.f.

⁸ It might, of course, be argued in general that as albumin chloride is a salt—its composition being a function of pH—it will act as a strong electrolyte and be completely ionized at least over the entire range of concentration contemplated in the measurements of Pauli and his collaborators. As regards the likelihood of attachment of Cl^- ion, i.e., the existence of undissociated saltmolecules, it would seem that localized adsorption in the Langmuir sense upon certain fixed charged places situated on a solid surface would be more clearly analogous to the conditions obtaining here than is the case of attachment of Na^+ to Cl^- , for example, to produce an isolated molecule of NaCl . If the albumin were present as a porous solid carrying local positive charges, the possibility of attachment of the Cl^- ions would scarcely be denied. There does not appear to be any essential difference between the opportunity afforded for attachment in this condition and that represented by dissolved protein units. The question has to remain open as the experimental tests which suggest themselves may be equally well accounted for, either by combination, or by alteration (decrease) in the activity of the Cl^- ion treated as otherwise uncombined. Pauli, Frisch and Valko assume complete ionization of the albumin chloride over the entire range of concentration of HCl (0 — 0.05 N) examined. On this basis they calculate the activity coefficient of the Cl^- ion which apparently falls very rapidly with increase in concentration. This they ascribe to a marked depressing effect exerted upon the activity of the Cl^- ion by the presence of the albumin as cation: they assume that the activity of the H^+ ion is not affected by the albumin cation. In the account given above I have preferred to regard the albumin ion as not exercising any special influence upon the activity of the chloride ion. Instead it has been assumed that actual combination and consequently removal of a certain fraction of the Cl^- ions takes place. The activity of the free Cl^- ions is taken to be approximately the same as would obtain in a hydrochloric acid solution of similar concentration. The argument that the albumin ion by virtue of its very high valency would exert a depressing effect on the Cl^- ion activity may not be valid, owing to the circumstance that the protein ion is extremely large and the localized electrical effects may not exceed those exerted by a univalent ion. The view that the effective valency, so far as influence upon the activity of other ions is concerned, lies between one and two has been put forward by Simms (33) for the case of gelatin. Similarly Cohn and Prentiss (34) have concluded that crystalline hemoglobin acts as though it were divalent.

In the long run there may not be a very fundamental physical difference between the two views referred to since a marked decrease in activity is due to the operation of large interionic forces of attraction. When these are large enough one would speak of chemical combination in the ordinary sense. Some advantage, however, is probably gained by maintaining the distinction.

measurements of Bugarsky and Liebermann using hydrogen electrodes. No one, so far as the writer is aware, has as yet employed the sodium amalgam electrode to determine to what extent the sodium ion is combined at the same time. In the absence of such data one is forced to a consideration of the cryoscopic measurements of the above investigators, although it is recognized that in such a case as that considered the method is somewhat insensitive. Employing the data of Bugarsky and Liebermann we may proceed to make the following rough calculation. When 6.4 grams of albumin are dissolved in 100 cc. of NaOH which was originally 0.05 N the observed depression of freezing point, δ , is 0.097°. The albumin itself accounts for a depression (due almost certainly to electrolytic impurities) of 0.022°. Hence $\delta_{\text{corr.}} = 0.075^\circ$. This is due to the free Na^+ and OH^- remaining in the solution. From the e.m.f. measurements with the hydrogen electrode, Bugarsky and Liebermann calculate that with this quantity of albumin in alkali originally 0.05 N the hydroxyl ion is bound to the extent of 97 per cent of its original concentration. That is, the concentration of free OH^- ion is $0.03 \times 5 \times 10^{-2}$ equivalents per liter. Denoting the concentration of free Na^+ ion by x it follows that

$$\delta_{\text{corr.}} = 0.075^\circ = k \times 0.03 \times 5 \times 10^{-2} + kx$$

The factor k is obtained from the observation of Bugarsky and Liebermann that the 0.05 N NaOH alone gives a depression of 0.181°. Hence

$$k = 0.091 / 5 \times 10^{-2}$$

and

$$x = 3.95 \times 10^{-2} \text{ equivalents of } \text{Na}^+ \text{ ion per liter.}$$

Hence 21 per cent of the original Na^+ is combined with the protein. The same result is obtained for the case in which 12.8 grams of albumin were dissolved in 100 cc. of alkali. Although no stress can be laid upon the actual numerical value obtained which (in view of the fact that the depressions observed are

somewhat larger than would have been anticipated) may well be a minimal value, we are justified in concluding that an appreciable quantity of sodium ion is combined in the form of unionized protein salt at an OH^- ion concentration of $1.5 \times 10^{-3} N$ (i.e., $\text{pH} = 11.2$).

Hydrolysis of albumin chloride and sodium albuminate prepared from crystalline egg albumin, and the net average charge on the protein units at different pH values

The simplest type of hydrolysis of, say, albumin chloride, can be represented thus:



where Alb^+ denotes the cation form of albumin carrying the least possible charge, namely one; the symbol Alb denotes the iso-electric form of albumin. We are concerned with conditions not far removed from the iso-electric point. The existence of hydrolysis would thus be demonstrated by a calculation of the net charge on the albumin units at a given pH, a net value of less than unity denoting the existence of a certain number of uncharged (i.e., iso-electric) individuals present. If the net charge comes out greater than unity, the hydrolysis may be regarded as inappreciable.

From the titration data given by Loeb (35), one may calculate the equivalents of strong acid, i.e., of H^+ ion, combined with one gram of albumin in a 1 per cent solution of the latter existing initially at the iso-electric pH. Taking the molecular weight of albumin as 43,000, it follows that a 1 per cent solution is $1/4300$ or $2.3 \times 10^{-4} M$. Taking a particular case, Loeb finds that 1.15 cc. of 0.1 N HCl are required to bring the albumin from the iso-electric point ($\text{pH} = 4.8$) to $\text{pH} = 4.2$. This means that 1.15×10^{-4} equivalents of H^+ ion are bound. In other words, 1000 cc. of the albumin solution would bind 1.15×10^{-8} equivalents of H^+ ion. The ratio of equivalents of H^+ ion bound to the gram-molecules of albumin present is $\frac{1.15 \times 10^{-8}}{2.3 \times 10^{-4}} = 5$. That is, there are on the average 5 single positive charges upon each

albumin unit. Hence even at pH = 4.2 the hydrolysis to the iso-electric form is probably negligible. Similarly it may be shown that at pH = 4.4, the average charge is 3.3. Even at this pH, therefore, the quantity of iso-electric albumin is negligible. An analogous result is obtained from the titration data with alkali. Thus from Loeb's curve (*loc. cit.*, p. 60) at pH = 5, the net (negative) charge per molecule of albumin is 2.2.

It seems legitimate to conclude therefore that the concentration of iso-electric albumin is quite negligible compared with the ionic form over any pH range except in the immediate neighborhood of the iso-electric point, a range so small—namely of the order pH = 0.02—that for most purposes it could be regarded as sensibly identical with the iso-electric point itself.

It is of some interest to collect in tabular form the pH values, on either side of the iso-electric point, at which the electrical charges on egg albumin—in one case positive, in the other negative—are the same. See table 2.

It may be mentioned that the variation in the electric charge with pH given in table 2 is similar to the variation in the rate of electrophoresis exhibited by egg albumin according to the measurements (by the ultra-violet absorption method) of Svedberg and Tiselius (36), as would be expected if the magnitude of the net or "free" charge is the main factor influencing the rate of motion of the particles in the field.

3. Behavior of denatured protein with respect to acid and alkali

In the first place, it has been shown by Booth for egg albumin that the act of denaturation is not necessarily accompanied by any change in the number of titratable acidic and basic groups in the protein molecule. The essential matter in such titration experiments is to be certain that during the time taken for titration to the different pH values, the protein shall remain undenatured. This is possible by making use of the data of Cubin upon the rate of denaturation at various temperatures and at various pH values. The point is considered at length in the paper by Booth referred to above. The conclusion regarding the behavior of egg albumin is almost certainly true also for hemoglobin.

In the absence of neutral salt, the denatured proteins referred to remain in solution even in the neighborhood of the iso-electric point. When the solution is made distinctly acid or alkaline the denatured protein exists in solution in the form of protein salt. Probably the same relations hold for the ionization of this salt—the composition of which is a function of pH—as hold for the corresponding salt of undenatured protein. The experiment has not actually been carried out.

As will be pointed out later we have reason to believe that gelatin and caseinogen—in view of their method of preparation—more closely resemble a denatured than an undenatured protein. In view of this, it is pertinent to recall the behavior of caseinogen in the pH region 6–7, that is, on the alkaline side of its iso-electric point, where it exists in the form of caseinogenate anion.

TABLE 2
Positions of equal net charge (+ or -) for egg albumin

pH (POSITIVELY CHARGED UNITS)	pH (NEGATIVELY CHARGED UNITS)	MAGNITUDE OF MEAN NET CHARGE
4.8	4.8	0
4.2	5.4	5
3.8	6.3	10
3.65	7.0	12
3.4	8.0	15
3.1	9.2	20
2.8	9.9	25

Actually in the experiment about to be referred to, the protein system was examined in the presence of NaCl and CaCl₂, respectively. We are not here concerned with the question of union of both ions of the inorganic salt with the caseinogen individual,⁹ but with the degree of ionization of sodium caseinogenate and calcium caseinogenate respectively.

The investigation referred to is that of Wright (37), who investigated the Donnan distribution equilibrium (across a cellophane membrane permeable to simple ions but impermeable to caseino-

⁹ The effect of neutral salts upon proteins is considered in the next section. The experimental conditions referred to here were not adapted (nor were they so intended) to determine the union of chloride ion with caseinogenate ion, which at best is not an effect of large magnitude.

gen and caseinogenate anion) for the systems sodium caseinogenate-sodium chloride, and calcium caseinogenate-calcium chloride. The pH of the solutions remained between 6 and 7. The concentration of caseinogen was 0.5 per cent, this being dissolved in 0.025 *N* NaOH. On the other side of the membrane the initial concentration of NaCl was varied from 0.0125 *N* to 0.1 *N*. In the experiments with calcium ion, the concentration of caseinogen was varied from 2 per cent to 7 per cent in the presence of Ca(OH)₂, which in turn was varied from 0.025 *N* to 0.125 *N*, the CaCl₂ on the other side of the membrane being varied initially from 0.0125 *N* to 0.025 *N*. Wright concludes that, in order to make the Donnan theory apply quantitatively as it should do in a case like this, it is necessary to assume that the sodium caseinogenate is ionized incompletely, namely to the extent of about 68 per cent, while calcium caseinogenate is ionized only to about 30 per cent. The results recall those of Bugarsky and Liebermann for undenatured protein in alkali. Wright's calculations are based on "concentration" considerations. How far these figures would be affected by substituting activities for concentrations (as should theoretically be done) it is difficult to say.

4. On the question of the extent of union of undenatured and denatured protein with neutral salts

We are considering in the first place the behavior of solutions of egg albumin believed to be undenatured. Again reference may be made to the work of Bugarsky and Liebermann (*loc. cit.*). Sodium chloride was added to dialyzed albumin solution so as to make the sodium chloride 0.05 *N*. The quantity of albumin was varied from 0.4 to 6.4 grams per 100 cc. No change either in the freezing point, or in the e.m.f. of a chloride ion concentration cell was produced by addition of the protein. It is to be concluded, therefore, that under the above conditions no detectable union of Cl⁻ or Na⁺ with albumin takes place. (Again, the cryoscopic measurements are to be regarded as less accurate than the e.m.f. measurements.) Unfortunately the pH was not actually determined, but we may probably assume it to be in the neighborhood

of 6—the value for distilled water as ordinarily prepared.¹⁰ Since these authors do not refer to any coagulation of the albumin occurring under these conditions of pH and neutral salt content, we may infer with some degree of certainty that the protein was in fact undenatured.

The results obtained by Bugarsky and Liebermann for egg albumin are contradicted by those of Oryng and Pauli (29), who, working with evidently greater care, conclude that there is union of Cl⁻ ion with serum albumin. The precipitate obtained by the use of saturated ammonium sulfate was pressed, redissolved and dialyzed against distilled water for eleven weeks; no mention is made of bacterial decomposition. As the temperature was that of the laboratory, we may infer with some confidence that the material was undenatured, although the actual rate of denaturation of serum albumin at different pH values has not yet been effected. To detect the union of Cl⁻ with albumin, Oryng and Pauli employ the e.m.f. method, using calomel electrodes to detect the free Cl⁻ ion concentration. Readings are taken with and without the addition of serum albumin to a calomel-KCl solution—as in fact was done by Bugarsky and Liebermann with egg albumin. Oryng and Pauli find—as is to be expected if the pH of the solution is slightly on the alkaline side of the iso-electric point—that a fraction of the mercurio ions unites with the protein thereby releasing Cl⁻ ions, which introduces a correction term (treated as a constant but actually a variable, being a function of the Cl⁻ ion content) into the final estimates of the chloride ion bound by the protein. Inspection of the data shows that the e.m.f. differences produced by addition of albumin amount to 1–2 millivolts, which is not much to go on particularly in the very complicated system employed. Nevertheless the e.m.f. readings with albumin present are consistently higher than those obtained in its absence. The KCl content is varied over the range 0.01 *N* to 0.05 *N*. The corresponding quantities of Cl⁻ ion bound by 1 per cent albumin are 4×10^{-5} to 2.7×10^{-4} equivalents of Cl⁻.

¹⁰ Kendall (38) found for distilled water which had been allowed to come into equilibrium with the CO₂ of the air, that at 18° the pH = 5.67, and at 25°, the pH = 5.69.

per gram of albumin. It is evident that the upper value recorded does not necessarily represent the maximum combination possible for Cl⁻ ion. No attempt is made to determine the amount of alkali metal bound, if any. Unfortunately no pH determinations are recorded. The prolonged dialysis of the protein against distilled water possibly brought this material to pH = 6 approximately, but it is uncertain whether this remains unchanged in the final system containing protein, calomel and potassium chloride. If the pH is on the alkaline side of the iso-electric point, as it certainly appears to be, union of an anion is a fact of profound importance. On making the solution definitely acid, it is found that the Cl⁻ ion bound is increased. This is to be expected, however, in view of the positively charged protein units thereby brought into existence. It is of interest to remark here that Oryng and Pauli find that glycine combines with Cl⁻ ion while urea does not. The limiting combination for glycine is in round numbers 5×10^{-3} equivalents of Cl⁻ per mole of glycine at the pH of the solution—presumably about the iso-electric point (pH = 6.08) of this amino acid. It is known that glycine under these circumstances is very largely in the zwitterion form, which thus evidently possesses combining capacity presumably for both anions and cations.

Limiting our consideration to the simple salts of the alkali metals, the conclusion which appears to be justified is that while undenatured albumin in the form of cation, i.e. at a pH definitely on the acid side of the iso-electric point, is capable of uniting with, say, chloride ion to an extent which varies with the pH and the same is true for albumin as anion in respect to, say, sodium ion, union of sodium ion and/or chloride ion with dissolved undenatured albumin, when the latter is in the near neighborhood of the iso-electric point, is less certain, though it probably occurs to a slight extent.

A question which suggests itself at this point is whether undenatured iso-electric albumin has a greater or less tendency to unite with the ions of neutral salts¹¹ than has denatured albumin.

¹¹ In general the union here contemplated would not necessarily mean the binding of salt anion and cation in equivalent amounts.

No data bearing upon this appear to be available so far as the alkali salts are concerned. Fortunately, however, Pauli and Matula (39) have examined the behavior of both undenatured and denatured serum albumin towards silver ion. The cell employed is:

Ag	AgCl, AgNO ₃ 0.1 N (constant concentration)	KNO ₃ saturated	AgNO ₃ + protein	AgCl	Ag
----	---	-------------------------------	--------------------------------	------	----

It is not clear why the simple silver-silver nitrate electrode was not employed. The silver nitrate of variable concentration to which the protein could be added covered the range 0.0025 *N* to 0.05 *N*. The (undenatured) albumin concentration was 0.7 per cent. The differences in e.m.f. readings produced by the presence of the protein are larger than those obtained in the KCl experiments of Oryng and Pauli. The solutions remained clear until a concentration of 0.04 *N* AgNO₃ was attained. With 0.07 *N* AgNO₃ the precipitation of the albumin was complete, "a heavy white precipitate" being obtained. Presumably this is silver albuminate; it does not appear to have been analyzed, nor is it stated whether it was crystalline or not.

As regards union of silver ion with the dissolved protein, Pauli and Matula point out that the same limiting degree of combination—amounting to 5×10^{-4} gram equivalents (? 6×10^{-4}) in round numbers per gram of albumin—is attained both by the undenatured and denatured, but still dissolved, protein. Nevertheless, there is marked difference in behavior between the two, for it is found that the denatured material reacts much more readily than the undenatured, *the denatured protein approximating towards the limiting degree of combination at a much lower concentration of silver nitrate than is the case with the undenatured protein*. This is in agreement with the view that at the pH obtaining in the experiments—once more its precise value is uncertain, but it is evidently on the alkaline side of the iso-electric point as indicated by cataphoresis experiments referred to below—the undenatured molecular units present a certain degree of obstruction to the

salt ions not exhibited by the denatured units. The obstruction slows down the rate of interaction without influencing its ultimate extent. It almost looks as though the undenatured molecule had a number of closed rings forming part of its structure, which are absent in the denatured form. The behavior of serum albumin appears to find a parallel in the behavior of gelatin and casein, two proteins which are not denaturable but which probably exhibit certain resemblances to a denatured protein. In these cases heating has absolutely no effect upon the extent of combination with silver, which, judging from the curves given in the paper of Pauli and Matula approximates to the maximum value for the individual protein at a lower concentration of silver nitrate than is the case with undenatured serum albumin.

Pauli and Matula point out that the limiting extent of combination with silver ion in equivalents is the same as that found by Oryng and Pauli for chloride ion in the experiments with KCl in the presence of calomel.¹² Evidently it is assumed that the pH is the same approximately in the two cases. That it lies on the alkaline side of the iso-electric point is shown by cataphoresis experiments, which indicate a negative charge on the "pure proteins" (gelatin and serum albumin) as well as on the silver compounds.

Measurements with undenatured egg albumin and $ZnCl_2$ (Pauli and Schön (40)) showed similar behavior to the silver nitrate case. The pH which was determined in this case indicates a H^+ ion concentration of the order 10^{-6} , corresponding to the alkaline side of the iso-electric point. Cataphoresis experiments showed the protein material to be negatively charged. The Cl^- ion, which is the important one in view of the pH value, is shown to be bound and evidently to a greater extent than in the case of KCl. We must conclude, I think, that Pauli has justified his contention regarding the capacity of a salt anion to combine with negatively charged protein. This being the case, the claim that no union occurs at the iso-electric point falls to the ground. Evidently

¹² This statement appears to be fairly exact for gelatin; the evidence is less certain for serum albumin. On the whole the Cl^- ion bound seems to be little less than the Ag^+ ion bound.

some union is possible for both ions at the iso-electric point and in the neighborhood on both sides thereof. Further confirmation along the lines suggested by Northrop and Kunitz (41) and carried out by them for gelatin would however be desirable for denaturable protein. Incidentally, the work of Pfeiffer (42) upon the isolation of compounds in the solid form between simple amino acids and neutral salts, i.e., both ions involved simultaneously, may be taken at least as evidence of the possibility of similar compounds with protein in solution.

The conclusion that both ions of inorganic neutral salts are capable of uniting simultaneously with protein in solution at or near the iso-electric point does not require of course that undenatured protein when crystallized out in the presence of such salt and subsequently washed should still contain the salt, for as Pauli has shown these compounds are reversible and the salt may therefore be removed beyond the limits of detection by washing. The conclusions of Pauli are therefore quite consonant with the finding of Sörensen already referred to regarding his inability to detect $(\text{NH}_4)_2\text{SO}_4$ in the washed crystalline egg albumin.

It has been tacitly assumed in the above that the Na^+ and Cl^- attach themselves to carboxyl and amino groupings respectively. There is no conclusive evidence that such is the case. Thus the experimental results obtained by Northrop and Kunitz (43), using a membrane p.d. method, regarding the union of various anions and cations with gelatin are for the most part difficult of interpretation as regards the position at which the addition takes place. For example, the conclusion, based upon Hitchcock's observation (44) to the effect that, since the deaminization of gelatin diminishes the amount of hydrogen ion combined (from 0.00089 moles of acid per gram of untreated gelatin to 0.00044 moles per gram of deaminized gelatin), the hydrogen ion therefore combines at the amino group, is not necessarily true. Thus deaminization by destroying a certain number of the free amino groups would cause, if the zwitterion form exists, a corresponding number of the "partner" $-\text{COO}^-$ groups to become practically unionized $-\text{COOH}$ groups, no longer titratable by acid of which a smaller amount is now required, corresponding, in fact, to the

zwitterion $-COO^-$ groups unaffected (indirectly) by the deamination. Obviously this way of regarding the matter entails the assumption that deamination does not remove all the free amino groups. Simms (45), who repeated Hitchcock's measurements, concludes that "deamination removes a large part of the lysine group and does not remove the pre-arginine, the arginine or the histidine groups."

An important observation made by Northrop and Kunitz is that the position of attachment of hydrogen ion is likewise the position of attachment of copper ion. As to the location of other ions the evidence is uncertain. In the case of Cl^- ion Northrop and Kunitz show that when $CuCl_2$ is employed the amount of Cl^- ion combined is the same whether the gelatin be deaminized or not. Of the two types of groups, amino and carboxyl, it is evident that Cl^- would only combine at an amino group. We might possibly account for the above result by asserting that the Cl^- unites at the pre-arginine, arginine or histidine portions and not at the lysine. This would be purely an *ad hoc* assumption at this stage. Failing this, we would have to assume union at some other group, such as the peptide linkage or the "oxonium" oxygen of the carboxyl group. If this be assumed in this case, it would be only logical to take such positions into consideration when union with cations is considered. This would greatly enlarge the variety and therefore complexity of the problem presented by union of protein with simple ions. The position is therefore very obscure at present.

As will have been realized the real difficulty in demonstrating beyond any doubt the fact of union and the extent of union of both ions of a neutral salt with a protein at its iso-electric point lies in the minuteness of the extent of union. This is particularly the case with salts of the alkali metals. Parenthetically it may be well to point out that the union of either or both ions of a neutral salt with iso-electric protein even on the assumption (which may not be true) that union takes place at the amino and carboxyl groups is not at variance with the conclusion already drawn regarding the behavior of protein towards dilute acid alone or dilute alkali alone, resulting in the production of a largely ionized

protein chloride and sodium proteinate. These are true salts, and as such are largely ionized over a fairly wide pH range and completely ionized in the neighborhood of the iso-electric point, i.e., no union of protein ion with oppositely charged inorganic ion. The amount of the latter present from the acid or alkali is small in the neighborhood of the iso-electric point (of the order $10^{-4.7} N$) compared with the concentrations of the ions of neutral salt (of the order 0.01 to 0.1 N) employed in the experiments dealt with above.

Ito and Pauli (46) have recently investigated the effect of neutral salts upon a number of proteins on the alkaline side of the iso-electric point. They find that with salts of the NaCl type even up to 0.02 M the direction of motion (of egg albumin and serum albumin) is unchanged—namely, towards the anode. With chlorides of Ba⁺⁺, Ca⁺⁺ and Mg⁺⁺ at 0.05 M , migration occurs in both directions. In the case of gelatin the chlorides of the alkaline earths reverse the direction of motion of the protein particles even at a concentration of 0.004 M .

Ito and Pauli have likewise examined the behavior of pseudo globulin on the acid side of the iso-electric point. They find that potassium salts containing the following anions: Cl⁻, SO₄²⁻, Fe(CN)₆⁴⁻, Fe(CN)₆³⁻, change the cathodic migration, reversal of the direction being produced by Fe(CN)₆³⁻ at $5 \times 10^{-5} N$, by Fe(CN)₆⁴⁻ at $1 \times 10^{-4} N$, by SO₄²⁻ at $7.8 \times 10^{-4} N$, while Cl⁻ at 0.05 N causes movement in both directions. These effects are in general agreement with expectation.

Although Pauli has succeeded in comparing the interaction of silver nitrate with undenatured and denatured serum albumin under concentration conditions at which flocculation did not occur even in the case of the denatured protein, similar experiments do not appear to have been attempted in other cases, e.g., with denatured hemoglobin or egg albumin and alkali salts. Under ordinary circumstances on addition of neutral electrolyte even in small amounts, the outstanding phenomenon is flocculation, which is a maximum in the neighborhood of the iso-electric point and diminishes as the pH diverges from this region. This effect, therefore, seriously interferes with an examination of the

possible union of the ions of the salt with protein denatured but still dissolved. We are justified probably in inferring some degree of chemical interaction even here in the light of the behavior (already briefly discussed) of a protein such as gelatin, which we have already suggested to be analogous to a denatured protein rather than an undenatured one. Casein may also be cited as a protein which behaves in a like manner.

5. Alteration in pH as a consequence of union of protein with neutral salt

The conclusion that the ions of neutral salts (not necessarily in exactly equivalent amounts) can combine to a slight extent with protein at or near the iso-electric point raises the question as to the possibility of alteration in pH as a consequence of such union. We shall deal with this first on the assumption that the protein unit contains a certain number of zwitterion forms, and secondly from the point of view of the unionized form. If the zwitterion form is present, addition of ions of, say, NaCl in equivalent amounts will obviously leave the pH of the system unaffected. If the Na^+ is more combined than the Cl^- ion this will mean that in addition to a number of symmetric structures represented by $\text{Cl}\cdot\text{NH}_3\cdot\text{R}\cdot\text{COONa}$, there will be produced on certain of the protein units one or more structures of the type $^+\text{NH}_3\cdot\text{R}\cdot\text{COONa}$. This involves a destruction of the balance of the original zwitterion. If these structures remain intact they will not alter the pH, but will incidentally give a positive charge to the protein units even at the iso-electric pH. If, however, such units combine with OH^- ion, as it seems likely they would do in view of the weakness of the isolated charged amino group, the pH of the systems would be expected to decrease, and this should be observable in spite of the buffering action of the protein which has not interacted at all with the salt.

Incidentally, the differential effect should become more pronounced the greater the difference in valency possessed by the salt ions involved. If the protein be denatured, flocculation would of course occur, and this possibly by the union of the positively charged individuals (as the case may be) with free

protein molecules. This point is considered in the section on flocculation and will not be discussed here. On the other hand, if the basic-acidic groups of the protein do not give rise to the zwitterion structure, interaction of Na^+ and Cl^- would entail the splitting off of H^+ and OH^- , respectively, from the carboxyl and amino groups. Once more, if exactly equivalent amounts of Na^+ and Cl^- are united, the pH will remain unaffected. If there is an excess of Na^+ combined, the solution should become slightly acid. As might have been anticipated, it is not possible therefore to decide on such evidence as this whether the zwitterion form is present or not. The only experimental work bearing upon the question of production of acidity or alkalinity as a consequence of union with neutral salts which is known to the writer is that of Chick and Martin¹³ (47), who have found, for example, that addi-

TABLE 3
Effect of addition of NaCl to a solution of crystalline egg albumin

CONCENTRATION OF NaCl	H ⁺ ION CONCENTRATION (DETERMINED ELECTRICALLY)
0	$10^{-4.88} N$ ($132 \times 10^{-7} N$)
0.1 N	$10^{-4.88} N$ ($132 \times 10^{-7} N$)
1.0 N	$10^{-5.30} N$ ($50 \times 10^{-7} N$)
2.0 N	$10^{-5.73} N$ ($19 \times 10^{-7} N$)

tion of considerable NaCl to a 1 per cent solution of crystalline egg albumin (practically at the iso-electric point initially) causes the solution to become alkaline (see table 3). Unfortunately the pH of the original NaCl stock solution itself is not recorded. As this may have been of the order 5.9, corresponding to aqueous solutions in equilibrium with the atmosphere, it is possible that the changes in pH indicated in the table were the natural consequence of increasing the NaCl content. On the other hand if the effect is a real one due to interaction of the protein and salt, the direction of the change indicates that the Cl^- ion is preferentially bound.

¹³ Chick and Martin themselves point out that the earlier work by Hardy and by Pauli and Handovsky upon alteration in pH by addition of neutral salts is rendered somewhat uncertain by the fact that the indicator method was employed. Chick and Martin use the e.m.f. method.

If we assume for the time being that the Cl^- ion attaches itself to an amino group of a zwitterion structure, such union will render the carboxyl ion free to unite with H^+ (from the water) with consequent formation of OH^- . If the amino and carboxyl groups are both in the classical form, addition of Cl^- at the amino $-\text{NH}_2\text{OH}$ group would necessarily liberate OH^- from that position directly.

It may be pointed out that from Loeb's titration data for albumin with caustic soda it follows that approximately 1.0 cc. of 0.1 N NaOH is required to bring 1 gram of albumin (dissolved in 100 cc.) from the iso-electric point to $\text{pH} = 5.3$. That is, 1×10^{-4} equivalents of OH^- are involved. This ought to represent in the sodium chloride case the excess of Cl^- ion bound by 1 gram of albumin over the Na^+ ion bound at this pH. It gives us therefore a lower limit for the quantity of Cl^- bound. The result is at least consonant with the finding of Pauli and Matula (already referred to), who estimate the upper limit of Cl^- bound by 1 gram of albumin as 5×10^{-4} equivalents, at a somewhat uncertain pH on the alkaline side of the iso-electric point.

In the second of the papers referred to, Chick and Martin have recorded the behavior of sodium citrate with respect to 0.057 per cent denatured egg white. The protein material is dispersed in acid to begin with. The concentration of sodium citrate employed was varied from 0.0002 N to 0.006 N. Flocculation manifested itself when the concentration of sodium citrate reached 0.0006 N. On raising the concentration to 0.004 N, dispersion once more took place. The initial pH of the system (sodium citrate 0.0006 N) was found to be 3.59; the final pH (sodium citrate 0.006 N) was 6.21. In the first solution the protein particles were positively charged, as would be expected in view of the appreciable acidity. In the final solution the protein is negatively charged. As the final pH is as high as 6.21, this of itself would cause the protein portion which has not reacted with the salt at all to take on a negative charge. The production of OH^- ion is evidently occasioned by preferential union of citrate ion. If, again, this is regarded as taking place at the amino group, an equivalent of OH^- will be produced, thereby increasing the pH as in the NaCl case. It is evident, however, from the magnitude

of the change in pH that citrate ion—as would be expected from its valency—is much more readily bound than is Cl^- ion. This capacity for union, coupled with the fact of a valency greater than unity, is also probably the cause of the initial flocculation at the iso-electric pH, followed by dispersion owing to the existence of numerous mutually repelling structures of the type $\text{Citrate-NH}_2 \cdot \text{R-COOH}$, i.e., divalent units. Owing to the probability of such units it is impossible to say whether the final negative charge is to be ascribed mainly to such or mainly to protein anions resulting from interaction with hydroxyl ion. Experiments in buffered systems would seem to be necessary in this connection.

Results have likewise been obtained by Chick and Martin for the salts KCl , K_2SO_4 and CaCl_2 acting on 1 per cent pure crystalline egg albumin, starting from a definitely alkaline point, $\text{pH} = 8.88$. Addition of these salts increases the H^+ ion concentration under the above circumstances. It would therefore be expected that preferential union with the cation of the salt occurs with consequent production of H^+ . Thus, with KCl at 0.05 N the pH alters to 8.42; with CaCl_2 at the same normality the pH alters to 7.87. It may be inferred that higher concentrations of CaCl_2 would further affect the hydrogen ion, i.e., increase it still more. It may be anticipated that a pH identical with that of the iso-electric pH of the pure protein could thereby be attained. Under such conditions, however, the protein would not be simple iso-electric material represented by the structure $\text{NH}_2 \cdot \text{R-COOH}$ or $^+\text{NH}_3 \cdot \text{R-COO}^-$, but material containing in addition a considerable number of structures of the type $^+\text{NH}_3 \cdot \text{R-COO}\text{Ca}^+$ as well as of the type $\text{Cl} \cdot \text{NH}_2 \cdot \text{R-COO}\text{Ca}^+$, the pH being no longer that corresponding to electric neutrality of the protein material as a whole. On further addition of CaCl_2 with alteration of pH to the acid side of the iso-electric point of the parent protein, it may be expected that preferential union with Ca^{++} will more or less rapidly diminish owing to the cation form of the normal protein coming into existence. Thus Northrop and Kunitz (*loc. cit.*) have measured by the membrane p.d. method the amount of Ca^{++} bound at different pH values in the case of gelatin, and have found that at pH values lower than 3 the Ca^{++}

is completely displaced by the H^+ ion. A similar behavior may be anticipated with albumin. The corresponding membrane p.d. measurements have not yet been carried out.

We may at this point draw attention to the apparently "antagonistic" effects produced by NaCl and KCl, respectively, on crystalline egg albumin as recorded by Chick and Martin. The alteration in pH in the two cases is such that we have inferred preferential union of Cl^- ion over Na^+ in the case of NaCl, but preferential union of K^+ over Cl^- ion in the case of KCl. It is highly improbable, however, that there can be any distinction of this sort as between potassium and sodium. The explanation would appear to be that a different pH range was employed in the two cases. Thus in the KCl experiments the initial pH, namely 8.88, is distinctly on the alkaline side. This condition will naturally be very favorable to union of a metallic cation since the protein units here carry a considerable negative charge. Consequently the preferential union of K^+ which is observed decreases the alkalinity, i.e., decreases the pH. It seems likely that at a pH not far from the iso-electric point but on the alkaline side thereof, the union of either K^+ or Na^+ would be just about the same as that of Cl^- ion. Such a position should correspond to union of both ions of salt in equivalent quantities without alteration in pH. This position has not yet been found experimentally, so far as I am aware. For Ca^{++} a similar position corresponding to equal union of both ions would be expected to lie on the acid side of the iso-electric point.

It follows further that the presence of a suitable inorganic salt with multivalent anion may cause part at least of the protein to carry a negative charge even on the acid side of the iso-electric point. This has been shown to be the case by direct electrophoresis measurements by Chick and Martin in the case of denatured serum protein in the presence of 0.07 N Na_2SO_4 at a pH = 4.01, the protein being in the dispersed state under these conditions. The excess union of SO_4^{--} thus indicated, naturally demands that prior to addition of the salt the pH of the solution must have been more acid still. This is the case, the initial pH being 3.24, at which point it was shown by experiment that the protein was dispersed and positively charged.

In the light of the above considerations we are probably justified in concluding that observed alterations in pH—a quantity susceptible of very accurate determination—afford the most exact means at present available for determining the difference in the extent of union of the salt anion and cation respectively. If one of these ions can be determined with accuracy by an independent method, say the Na^+ ion by means of the sodium amalgam electrode, the problem of the extent of union of both ions would conceivably be brought in such a case to a much more satisfactory position than exists at present.

Returning to the results obtained by Chick and Martin for the alteration in pH on addition of CaCl_2 , it is of interest to calculate the extent of the differential effect indicated thereby under the condition of the experiment. Thus, if x is the number of equivalents of Ca^{++} bound by 1 gram of protein in excess of the amount of Cl^- bound simultaneously, then x is likewise the net number of equivalents of H^+ which are thereby produced, and if y is the number of equivalents of H^+ taken up by the residual protein to reduce the negative charge, then $x - y$ is the number of equivalents of H^+ effectively added to the solution and remaining as such. In the experiments referred to it is found that the H^+ ion concentration increases from $10^{-8.88} N$ to $10^{-7.87} N$ by the addition of CaCl_2 in such quantity as to make the solution $0.05 N$ with respect to this salt. The increase in H^+ ion concentration observed is 0.1217×10^{-7} gram-equivalents per liter. Since the volume of the solution employed is 100 cc. (this volume containing 1 gram of crystalline egg albumin), the increase in mass of H^+ in equivalents is 0.1217×10^{-8} . That is $x - y = 1.2 \times 10^{-9}$ equivalents. From Loeb's titration curve it follows that approximately 0.9 cc. of $0.1 N$ NaOH is taken up by 1 gram of albumin in 100 cc., when the pH of the solution is changed from 7.87 to 8.88. Hence this quantity of $0.1 N$ acid would be taken up by the albumin in reversing the titration. That is, $y = 9 \times 10^{-5}$ equivalents. It follows that x and y are practically identical, *viz.* 9×10^{-5} equivalents. Hence when 5×10^{-8} equivalents of Ca^{++} are present in 100 cc., 9×10^{-5} equivalents of Ca^{++} are bound by 1 gram of the protein at $\text{pH} = 7.87$, in excess of the amount of Ca^{++} .

bound simultaneously with and balanced by Cl^- ion. If the latter be very small, as would be expected at a pH so alkaline as 7.87, then the above figure gives the (minimum) value of Ca^{++} bound under these conditions. It may be mentioned in this connection that, according to Northrop and Kunitz, in the case of gelatin the amount of Ca^{++} bound attains a roughly constant value on the alkaline side of the iso-electric point, amounting to 3.5×10^{-4} moles per gram.

As already mentioned, addition of KCl to crystallized egg albumin in the distinctly alkaline region reduces the alkalinity. In view of this behavior it might be thought that the function of the inorganic salt in bringing about flocculation of the protein is that of adjusting the pH of the system towards the iso-electric point. It will be recalled, however, that in the case of NaCl, addition of the salt at pH = 4.88 altered the pH away from the iso-electric point. Such alteration therefore cannot account for the flocculating action. As Chick and Martin express it, "Whatever may be the nature of the process involved, it is clear that the change in reaction of protein solutions caused by electrolytes is inadequate to explain the influence of the latter upon agglutination." A possible mode of interaction in this connection is considered in the section dealing with flocculation.

A striking illustration of the effect of alteration in pH produced by a salt is afforded by the results of Thomas and Norris (48) in connection with the effect of heavy metal salts, namely, zinc chloride and thorium chloride, upon egg albumin. In this case, however, the increase in H^+ ion is ascribed entirely to hydrolysis of the inorganic salt itself. The very large variation in H^+ ion thus obtained is shown by the fact that with ZnCl_2 at 0.0008 M the pH = 7.07, at 1.715 M the pH = 2.5. Thomas and Norris, as a matter of fact, are concerned with the precipitating action of such salts, especially with the existence of the "tolerance region" between the first and second zones of precipitation. It is concluded that the effects to be expected are a function of the H^+ ion concentration, the first precipitation (with low salt content) being due to union of the heavy metal cation with albumin in the form of anion to produce a reversible precipitate. With further

addition of inorganic salt, the H^+ ion passes to the acid side of the iso-electric point with the result that the metal-albumin salt is no longer formed. On greatly increasing the salt concentration the albumin is denatured and is apparently regarded as being precipitated as such, though it may well have been denatured albumin chloride. (Compare Chick and Martin's results with denatured albumin sulfate, considered in the section on flocculation.) It is concluded that a "heavy metal ion is intrinsically an albumin denaturant." In general, the explanation offered for the tolerance zone is in agreement with the general line of thought pursued in the present section. As to why or how a heavy metal ion is capable of bringing about denaturation nothing is known. On the supposition that it is really a hydrogen ion effect it would be necessary to assume interaction of heavy metal even with the positively charged protein unit with local increase in H^+ ion. For this to occur it would be necessary to show actual union with the metal by means of cataphoresis. This was not done in the above case.

6. Solvent action of neutral salts and of non-electrolytes upon undenatured and denatured protein

We are not dealing here in the first instance only with proteins such as albumin and hemoglobin which possess an appreciable solubility in water, but with proteins of the globulin type which are insoluble in water but "soluble" in neutral salt solution. When much salt is present it acts as a precipitant, e.g., ammonium sulfate.

This striking, and as yet, imperfectly understood behavior has been the subject of considerable investigation at the hands, amongst others, of Hardy (49), Mellanby (50), Sørensen (51), Osborne and Harris (52), Cohn and Prentiss (53). The amount of serum globulin "dissolved" by a neutral salt depends not only upon the nature and concentration of salt but likewise upon the amount of protein present in the original globulin suspension. This has been ascribed to the existence of more than one protein

in the material employed.¹⁴ It should perhaps be emphasized that the concentration of salt here contemplated is large compared with that required to bring about flocculation of a denatured protein of the egg albumin type. The phenomenon, in fact, suggests peptization rather than solution proper. So far as I can gather, the concentration of salt which possesses solvent action is comparable with the upper limits of concentration which have been employed in connection with experiments on the union of neutral salts with proteins of the albumin, hemoglobin and gelatin types.

Hardy (*loc. cit.*) has drawn attention to a striking quality of globulin when dissolved in neutral salt. "The solution contains no ionic, no electrically charged globulin, as is shown by the absence of all movement in an electric field, while solutions in dilute acids and alkalies are ionic." It would seem that both ions of the salt are in combination with the globulin to equivalent extents.

To give an idea of the extent of solvent action produced by salts, reference may be made to some of Mellanby's experiments with KCl acting upon globulin, from horse serum, at 20°, the globulin being initially in the form of a suspension in water containing approximately 1.4 grams of protein in 100 cc. The solvent action is expressed as "per cent of original globulin dissolved" by the salt. With KCl at concentrations 0.047 *N* and 0.0806 *N* respectively, the "per cent of original globulin dissolved" increased from 40.7 to 84.0. With NaCl the increase in solubility expressed in the same manner rose from 35.7 to 91.5 on altering the concentration of NaCl from 0.0427 *N* to 0.0853 *N*. Incidentally, Mellanby finds that the solvent action of potassium iodide is greater than that of potassium bromide and this is in turn greater than that of potassium chloride, but the differences are not large.

In the case of the vegetable globulin edestin, Osborne and Harris (*loc. cit.*) give a considerable number of solubility deter-

¹⁴ The effect of the mass of globulin upon the composition of the fluid phase was first detected by Hardy (54) who, suspecting it to be due to a second component, repeated the experiments with "pure crystals of edestin washed with 20 per cent alcohol." The phenomenon was again observed. It is evidently a real characteristic of globulin behavior.

minations with salts of different valency and concentration. With NaCl at concentration 0.4 *N*, the solubility of edestin is 0.5 gram in 100 cc.; with 0.8 *N* NaCl, the solubility is 7.5 grams in 100 cc., the increase in solvent action being very great in the case of edestin. Bivalent salts are more potent agents than univalent salts.

That the solvent action is concerned with some kind of excessive disaggregation of the "solid" globulin is indicated by the finding of Gortner, Hoffman and Sinclair (55) in connection with certain vegetable globulins to the effect that on dissolving the protein with the aid of KBr (a very effective agent) and subsequently dialyzing so as to obtain the solid once more, this solid is now much more readily peptizable by KCl than it was in the first instance.

While it is almost certainly correct to describe the solvent action as peptization, this of itself does not carry us very far as regards the mechanism of the process, which still remains one of the least understood in the field of the physical chemistry of the proteins.

As regards the mechanism of the peptizing or solvent action of neutral salts in appreciable concentration upon globulins it appears to be generally assumed that the effect is a "direct" one due to the interaction of the salt (ions) with the protein. That peptization may be "indirect", that is, may have its origin in an alteration in the relative amounts of monohydrol and dihydrol of the water, has been suggested by Bancroft (56).

It should be mentioned that, according to Mellanby, "globulin is precipitated from solution in neutral salts by minute quantities of salts of the heavy metals."

Mellanby has concluded that "solution of globulin by a neutral salt is due to forces exerted by its free ions. Ions with equal valencies, whether positive or negative, are equally efficient and the efficiencies of ions of different valencies are directly proportional to the square of the valencies." The latter statement does not as yet rest upon a very certain experimental foundation. The position of the problem up to 1925 is well described by Cohn (57). Regarding the concentration of salt

involved, Cohn writes, "Many times as much salt as acid or alkali is required to dissolve a globulin, and solubility bears a logarithmic rather than an arithmetic relation to concentration." Clearly we are no longer dealing with stoichiometric effects alone, such as have come under review in previous sections.

Such stoichiometric unions must of course be presumed in the present case in the light of the behavior of the water-soluble proteins albumin and gelatin with respect to neutral salts. The acceptance of this, however, does not afford any real explanation of the solvent action itself, which has to be sought on a non-stoichiometric basis as essentially due to a change in environ-

TABLE 4
Solvent action of salts upon proteins
(Cohn and Prentiss)

pH = 6.8

CONCENTRATION OF PHOSPHATE $\text{KH}_2\text{PO}_4 + \text{K}_2\text{HPO}_4$	SOLUBILITY OF HEMOGLOBIN PER 100 CC. <i>grams</i>
0.0213	3.32
0.062	3.81
0.121	4.85
0.293	6.66

ment. Incidentally, one would expect that the conditions for stoichiometric union would be most favorable subsequent to the disaggregating or peptizing effect itself.

Although the solvent action of salts upon proteins has been most clearly recognized in the case of the globulins, owing to the fact that these are practically insoluble in water, a similar effect would be expected even in the case of substances such as albumin and hemoglobin which are soluble to some extent in water, provided they be undenatured. This has been observed by Cohn and Prentiss (*loc. cit.*) for crystalline oxyhemoglobin from the horse, using a buffer to fix the pH. Table 4 illustrates the order of magnitude of the effect, which is much greater than would have been anticipated, especially as the concentrations of salts employed are by no means great. We are

evidently dealing with a phenomenon essentially similar to that observed in the case of the globulins. The solubility at pH = 6.6 was less than at either 6.4 or 6.8. Cohn and Prentiss treat the effect in terms of the Debye theory, according to which the activity coefficient of a given ionic species is diminished by the presence of all the ions existing in the solution. If the solubilities of hemoglobin in water alone and in a salt solution are S_0 and S respectively, it follows on the basis of the theory of activity that $\alpha_0 S_0 = \alpha S$, where α_0 and α stand for the activity coefficient of the protein in water and in the salt solution respectively. Since the activity coefficient α_0 can be set equal to unity in such a case, it follows that

$$\log_{10} \frac{S}{S_0} = - \log_{10} \alpha$$

Denoting by c the concentration of any single ionic species (say the Na^+ ion or Cl^- ion in the case of NaCl solution) and by v the valency of this ionic species, then Σcv^2 stands for the sum of the cv^2 terms for all the ionic species present. Cohn and Prentiss employ the equation of Debye, which expresses the logarithm of the activity coefficient of a given ionic species—the hemoglobin in the present case, here treated as an electrolyte with mean valency z —in terms of Σcv^2 , which measures the influence exerted by the ions upon the protein activity coefficient. The equation is

$$-\log_{10} \alpha = \log_{10} \frac{S}{S_0} = \frac{0.5 z^2 \sqrt{\Sigma c v^2}}{1 + 1.5 \sqrt{\Sigma c v^2}}$$

Cohn and Prentiss show the general applicability of this expression to dissolved horse hemoglobin, by obtaining with its aid a reasonably constant value for S_0 , the magnitude of which is in agreement with the somewhat approximate but directly observed value in water alone. To make the equation fit in this satisfactory manner, it is necessary to conclude that hemoglobin acts like a bivalent or uni-quadrivalent compound, i.e., $z = 2$.¹⁵

¹⁵ The authors suggest that the second alternative is physically the more probable in the present case. It may be added that the measurements referred to apply to a pH region near the iso-electric point. It is probable that the valency is a function of pH.

With the aid of the equation the values of S_0 are calculated to be 12.2, 11.2, and 13.1 grams per liter at pH 6.4, 6.6, and 6.8, respectively. The authors point out that the effect of salt on hemoglobin, though very considerable, is much less than upon a globulin. They regard the latter type of protein as presumably of higher valence type. In the case of edestin $\sqrt{z^2}$ would have to lie between 5 and 6 to account for the solubility data of Osborne and Harris on the basis of the Debye theory of ionic interaction.

Incidentally, the change in solubility of hemoglobin with pH serves to indicate that this is a factor the possible significance of which has been overlooked hitherto in investigations of the solvent action of neutral salts upon globulin suspensions. It may be pointed out that the fact that hemoglobin shows minimum solubility—which on the above mode of treatment one would take as indicating minimum valency, i.e., an iso-electric state—not at the true iso-electric point but at a pH on the acid side thereof, is probably to be ascribed to preferential union of protein with the phosphate ion present in the buffer employed, thereby giving the protein unit an effective negative charge which is balanced by an alteration to a more acid environment.

It must be pointed out that to ascribe to z , the valency of the protein, a value greater than zero implies that the protein has certain groups effectively electrically charged even at the iso-electric point. This point has already been made by Bjerrum himself (58) in connection with simple amino acids. Bjerrum suggests that a mode of demonstrating the existence of zwitterions, as distinct from the classical form, would be to determine the influence of electrolytes on the activity coefficient of the amphoteric body. To quote from Bjerrum's paper: "For the form $\text{NH}_2 \cdot \text{R} \cdot \text{COOH}$ it is to be expected that the solubility will not be influenced by addition of neutral salt to a greater extent than the solubility of a non-electrolyte in general would be influenced. It is otherwise for the form $^+\text{NH}_3 \cdot \text{R} \cdot \text{COO}^-$. In view of the electric charges one would expect that an addition of neutral salt would here diminish the activity and consequently increase the solubility, as is always the case with salts not having an ion in common. If the two charges are far apart, the

increase in solubility ought to be twice as great as for an ordinary univalent salt. The closer the charges are together the smaller must be the effect, since the charges partly annul one another's influence. Preliminary solubility measurements of methyl orange in potassium chloride solutions . . . indicate an effect which is about one-third of that expected on the basis of distant charges." Under the conditions of the experiments referred to methyl orange exists almost entirely in the zwitterion form. Bjerrum likewise points out, "the zwitterion of an amphoteric electrolyte like all other ions must likewise diminish the activity of other ions present." He finds this borne out by experiment. Further, "mixtures of amino acids are much more soluble in water than would be expected from the solubilities of the single acids. Not only do the acids crystallize with greater difficulty, but they are in fact more soluble. Thus the unexpected increase in solubility on the part of difficultly soluble substances of this type can be explained by their zwitterion nature as arising from attractive forces between the electric charges." The mutual effect here referred to by Bjerrum may possibly be operative in biological fluids, for example in blood, where globulin and albumin are present simultaneously.

In spite, however, of the apparent cogency of Bjerrum's argument, the possibility still presents itself that the strongly dipolar groups represented by unionized amino and carboxyl might be as effective as zwitterions in respect to the influence of salt and other substances on solubility.

To return to the question of the effective valency of a protein as estimated from the influence of neutral salt upon the solubility of the protein, it may be recalled that in the work referred to undenatured protein was employed. It would be of considerable interest to attempt to make similar measurements with denatured material as a step towards understanding the peptizing effect to which appeal has to be made in considering the solvent action of salts upon insoluble proteins, e.g., the globulins.

A further point arises in connection with the application of the Debye equation to the solvent action of salts upon a protein. The dielectric capacity D appears in the numerator and in the

denominator of the Debye expression, the numerical factor 0.5 (in the form of the equation quoted above) including a group of terms having $D^{3/2}$ in the denominator thereof, the factor 1.5 likewise having $D^{1/2}$ in the denominator of the group of terms covered by this numeral.

As ordinarily employed the value used for D is the dielectric constant for water at the temperature to which the measurements refer. With the concentrations of salts present in protein solubility determinations referred to, the D term may differ somewhat from that of pure water. Dielectric capacity measurements of aqueous salt solutions carried out by different observers show very great discrepancies. On the whole there is agreement as to the fact that on addition of small quantities of salt the dielectric capacity falls. On further addition of salt, beyond the concentration 0.01M approximately, according to the observations of Schmidt (cf. Carman and Schmidt (59)), the dielectric capacity in the case of NaCl and KCl rises and soon exceeds that of water. In the latter region (which may correspond with that employed in the experiments under consideration) to make the Debye equation apply it would be necessary to adopt higher values of z (the valency of the protein) than that suggested by Cohn and Prentiss. The initial fall in the dielectric capacity on addition of very small quantities of salt would, if the Debye equation be applicable, be expected to increase the solvent action more rapidly than that calculated in the ordinary way. Actually this anticipation does not appear to be realized. Thus Osborne and Harris (*loc. cit.*) state that "edestin, dissolved in water with a minimal quantity of alkali, forms a solution which is abundantly precipitated by minute quantities of sodium chloride, in just the same way as solutions similarly made with acid are precipitated, the precipitate in each case being dissolved by a larger proportion of salt."

If this behavior is general in character¹⁸ it suggests that before the Debye effect becomes appreciable the effect of salts, in minute

¹⁸ Analogous results for the effect of salts on albumin have been obtained by Matthews (61).

amounts, is essentially that of inducing colloid precipitation.¹⁷ In other words, the presence of small amounts of salt acting by chemical union or by local decrease in dielectric capacity, or by the operation of both functions, "sensitizes" the colloidal particles or suspension thereby increasing the tendency towards aggregation.

In discussing the presumed alteration in dielectric constant attributed to the presence of added salt it is necessary to draw attention to an effect likewise involving dielectric capacity which may have a completely swamping effect as compared with any alterations in D produced by the salt. I refer to the effect of the protein itself upon the dielectric capacity of the solution. According to the measurements of Marinesco (60) upon methemoglobin (source, method of purification and pH not stated) an astonishingly great fall in the dielectric capacity of the "solution" in water occurs. Thus with 0.75 per cent methemoglobin $D = 58.3$, while with 2 per cent protein $D = 31.8$. Provided these dielectric capacity measurements are reliable, it would be inferred that for saturated solutions of methemoglobin (and therefore very probably for oxyhemoglobin) the numerical value of the group of terms collected in the numeral 0.5 in the numerator of the Debye equation, as well as one of the terms in the denominator would be so much altered as to give rise to too small values for z , the effective valency of the protein. The matter is raised at this point although it cannot be carried further owing to the complete lack of the necessary dielectric constant data.

Apart from the particular point just raised, it is evident that the Debye equation might be regarded as affording a basis for the increase in solubility exhibited by a protein by the addition of neutral salt, provided each unit of the protein carries effective electrical charges even though equal in number but of opposite sign. Such an effect is hardly to be distinguished from a peptization effect, which from this point of view would be ascribed to an electrical environmental effect. Any actual combination of either or both of the salt ions with the protein would on this basis be regarded not as a cause of peptization but merely as a concomitant. That the theory underlying the Debye equation cannot be regarded as covering the whole of peptization becomes

¹⁷ We are here anticipating to a certain extent the topic discussed in a later section, *viz.* flocculation.

evident when we recall that peptization of material, whether electrically neutral or not, may be brought about by non-electrolytes. Further, in using the term peptization we have in mind not only an increased solubility effect, but likewise a preliminary (and essential) disaggregation of gross units of colloidal material. This implies that the cohesive forces operative between the colloidal units of a large aggregate can be sufficiently reduced by the peptizing agent. The existence of this phenomenon suggests the possibility of the applicability of another expression, namely the "distribution" equation of Bjerrum and Larsson (62). This equation is intended strictly to apply to the solubility of an electrolyte in ion-free media of different dielectric capacities. At first sight, therefore, it would appear to be wholly inapplicable to the present case. It is just possible, however, that a complex "tree-like" structure such as a protein molecule or aggregate may be so bulky that in comparison therewith even a salt solution may be regarded as a medium essentially homogeneous and of uniform dielectric capacity. The act of dissolution must certainly involve the protein molecule as a whole, though it may be dragged into solution, so to speak, by weakening of the localized forces operative between certain points on the contiguous protein molecules, these forces being associated with a limited number of groups (possibly the amino or carboxyl groups in the zwitterion form) likely to possess marked mutual affinity. From this point of view it seems not unreasonable to regard the effective valency as comparable with the number of amino or carboxyl groups in the zwitterion form. This would mean a number varying with the pH of the solution, being of the order 2 to 5 presumably in the neighborhood of the iso-electric region. It is evident that these electrically charged regions are widely separated from one another in the protein molecule as a whole. At pH values sufficiently far removed from the iso-electric region, the effective charge on any protein unit becomes predominately of one sign, with consequent mutual repulsions between contiguous units and therefore additional solubility—in agreement with experiment—on this ground alone quite apart from dielectric capacity considerations. We are concerned for

the moment more particularly however with the behavior in the region of the iso-electric point.

The Bjerrum-Larsson equation deals primarily with the distribution coefficient or ratio of solubilities of an electrolyte—in the case considered, the protein—in two different environments. Regarding the change in solubility as due solely to the alteration in the electrostatic forces owing to alteration in dielectric capacity, the equation is

$$\log_{10} \frac{c_1}{c_2} = \log_{10} \frac{S_1}{S_2} - \frac{z^2 \times 1.2 \times 10^8}{r} \left(\frac{1}{D_2} - \frac{1}{D_1} \right)$$

where S_1 and S_2 denote the solubilities of the protein in two media of dielectric capacity D_1 and D_2 respectively. z is the mean valency or charge (of either sign) upon the protein unit, and r the radius of the protein unit expressed in Ångstrom units.

Taking 65,000 as an average molecular weight for a protein, and treating the molecule or unit as though it were a sphere of unit density, it follows that $r = 30\text{\AA}$. The value of z we shall consider as lying between 5 and 2. Whence the value of $z^2 \times 1.2 \times 10^8$

lies between 100 and 16. Taking a solution of 0.02 N NaCl as the first solvent, it follows from Schmidt's data that $D_1 = 93.6$.

With 0.016 N NaCl, $D_2 = 80.8$. Whence $\left(\frac{1}{D_2} - \frac{1}{D_1} \right)$ is approximately 1.7×10^{-4} . Hence $\log_{10} \frac{S_1}{S_2}$ lies between 0.17 and 0.027.

That is, $\frac{S_1}{S_2}$ lies between 1.48 and 1.06. From the graphs given in Mellanby's paper the increase in solubility of serum globulin in so far as it can be estimated at all for the NaCl range 0.016 — 0.02 N appears to lie between 20 per cent and 40 per cent, i.e. $\frac{S_1}{S_2}$ lies between 1.2 and 1.4. It is impossible to say whether there is substantial agreement or clear disagreement between the observed and calculated values, owing to the uncertainty of the dielectric constant data on the one hand and of the solubility data on the other. In the case of edestin, much more extensive

solubility data are available, but the concentration of salt employed is far beyond that for which any dielectric constant data are available. It may be well to emphasize here an essential difference between the Debye equation and the Bjerrum-Larsson equation in respect to the dielectric constant term. On the Debye equation the solubility of a protein would be expected to fall as the dielectric constant increases, i.e., $\log S \propto 1/D^{3/2}$ (omitting the κ term of the denominator). On the Bjerrum-Larsson equation the solubility would be expected to rise as the dielectric constant increases.

In both cases the "response" of the protein to the surroundings depends upon the protein having one or more electric charges. If the material be a non-electrolyte, neither equation is applicable. Furthermore, as already mentioned, the Debye equation is intended to take account of the effects due to the presence of ions upon a given ionic species, while the Bjerrum-Larsson equation is intended to apply to an ion-free medium.

If the latter equation is applicable at all to the solubility of a protein, it would follow that addition of substances such as glycine and urea which, according to Fürth (63) raise the dielectric capacity of water, should increase the solubility, while addition of sugar or alcohol, which lower the dielectric constant, should decrease the solubility. Comparing absolute alcohol with water as solvents, one would expect on the Bjerrum-Larsson basis that the solubility of a protein in alcohol should be very much less than in water. This is true for certain proteins, e.g., gelatin, albumin, hemoglobin, but there are many known, e.g., the prolamines and the vegetable proteins, which are much more soluble in alcohol than in water. The environmental change here contemplated is so large that the solvent action upon material in the truly undissociated forms, considered by Bjerrum and Larsson but not included in the equation employed and discussed above, may well predominate.

Reference has been made to the possible effect of addition of urea to aqueous systems containing proteins, the effect anticipated on the basis of the Bjerrum-Larsson equation being that

of an increase in solubility.¹⁸ In this connection a number of striking observations have been made by Ramsden (64) Spiro (65) and others.

Ramsden states that "globulins, caseinogen, acid and alkali albumin, copper albuminate, fibrin, and even heat-coagulated proteins swell up and dissolve in a saturated aqueous solution of pure urea." Also "coagulable proteins are not heat coagulable in its presence, but reacquire their coagulability when the urea is removed by dialysis." Ramsden's observations have been confirmed by Osborne and Harris for edestin (undenatured and crystalline). In water the edestin is practically insoluble. With addition of urea edestin gives "a clear and bright" solution.

The fact that swelling precedes the act of dissolution points to an effect of a physical character (primarily) upon the forces holding the constituent parts of gels together, i.e., the overcoming of the cohesion. Since the forces here involved may well be electrical in origin, it is possibly not too speculative to suggest that alteration in dielectric capacity due to the presence of the urea is an important factor in its effect. If this is so, it is of interest to see what kind of results may be obtained by applying the Bjerrum-Larsson equation.

To this end Booth, in the writer's laboratory, determined the solubility of hemoglobin—a British Drug Houses preparation which was found to be salt-free and on solution in water gave a solution of pH = 6.6 (very close to the iso-electric point)—in water and in 5 per cent urea at room temperature. By addition of a little alkali the pH of the water plus protein was brought exactly to that of the urea, the absolute value of the pH of which was determined to be in the neighborhood of 9.9. That it is essential particularly in the case of urea effects to make comparison at the same pH values was pointed out to the writer by Professor Ramsden. It was found that the solubility of the protein in 5 per cent urea solution was 1.52 times that in "water," that is the urea has

¹⁸ That urea may react and combine with a protein in a purely chemical (stoichiometric) sense is of course highly probable. We are concerned at present however with only one possible aspect of its behavior, namely, the "environmental" effect.

increased the solubility by 52 per cent. According to the data of Schmidt (66), the dielectric constant of a 5 per cent urea solution is 1.04 times that of water. Taking D_{H_2O} as 80, it follows that $D_{urea} = 83.2$. Furthermore, taking z , the valency of hemoglobin as lying between 2 and 5, and setting $r = 30 \text{ \AA}$, it follows from the Bjerrum-Larsson equation that the electrical environmental effect produced by 5 per cent urea should cause the solubility of the hemoglobin to increase between 2 per cent and 12 per cent. Apparently the environmental effect to be expected on the Bjerrum-Larsson basis is small compared with the total effect actually produced by urea. However, no attempt has been made—nor can be made at present—to take into account the possible influence which may be exerted upon the magnitude of the Bjerrum-Larsson effect by the change in dielectric capacity of the system induced by the protein itself, since there are no data available for the dielectric capacity of solutions containing protein and urea together. Thus if 5 per cent urea in this case increased the dielectric capacity by 4 per cent (as before), then on taking the dielectric capacity of a saturated hemoglobin solution in water to be not greater than 30—a maximum value in the light of Marinesco's results already referred to—the Bjerrum-Larsson equation would lead to an increase in solubility between 5 per cent and 34 per cent, the lower value being the more probable of the two. It would appear that the environmental influence taken account of by the Bjerrum-Larsson equation is only likely to account for a part and possibly not a very large part of the effect exerted by urea. It would be necessary, however, to take it into account in any attempt to deal quantitatively with the total effect.

In concluding this section, it may be well to point out that no attempt is made to come to a definite conclusion as regards the relative merits of the Debye equation and the Bjerrum-Larsson equation in respect to their applicability to protein behavior. It is probable that both may be of assistance in interpreting different aspects of this behavior under different environmental conditions. At the present stage an exact test of either equation is impossible owing to lack of precise information upon protein

solubility in various media, the number of electric charges on the protein unit, and the dielectric capacities of aqueous solutions of salts and non-electrolytes.

III. FLOCCULATION

In this section we shall restrict ourselves almost entirely to the proteins of the albumin and hemoglobin type. As already mentioned, in the absence of salt the protein even when denatured may be maintained at the iso-electric pH without flocculation taking place. The coagulum when it is formed can be washed free from the neutral salt employed in its production. In other words, the ions of the salt do not form an essential feature of the structure of the amorphous colloid mass, the coagulum existing in virtue of cohesion operative between acidic and basic (probably carboxyl and amino) groups,¹⁰ although in the denatured but dissolved state these groups do not *spontaneously* unite with any measurable speed at ordinary temperatures.

There appears to be no doubt as to the effect exerted by small quantities of neutral salt in bringing about rapid and complete flocculation on heating. The position is less clear in the absence of neutral salt. The early work such as that of Aronstein (67) and of Heynsius (68) is contradictory and confusing. The most reliable information would seem to be that obtained by Chick and Martin (*loc. cit.*) who worked with crystalline egg albumin. Solutions of this material were dialyzed, and it is to be presumed that the dialysis was continued until the material was really electrolyte-free, although no actual analytical data are given. With this material a milky solution was obtained on heating for 15 minutes in boiling water. The filtrate however contained protein, i.e., coagulation was incomplete. This behavior refers to the solution as prepared in distilled water. The pH is not recorded, but presumably it lay between 5 and 6. On adding hydrochloric acid and heating, Chick and Martin find complete flocculation possible, "provided the pH be carefully adjusted" to

¹⁰ That the coagulum formed at the iso-electric point can be redissolved by a sufficient addition of acid or alkali may be taken as evidence that the links between the molecules composing the coagulum are ionizable acidic-basic in character.

the immediate neighborhood of the iso-electric point. On addition of a small amount of salt, the pH range over which flocculation is complete is greatly increased. Thus Chick and Martin find that a 1 per cent solution of undialyzed (albumin) crystals containing 0.3 to 0.4 per cent ammonium sulfate "will coagulate perfectly on boiling over a wide range of acidity (hydrogen ion concentration $10^{-8.1} N$ to $10^{-7.4} N$)."

The usual explanation given for the favorable effect of heating is not only that the essential denaturation is brought about, but also that the protein salt of the acid, when such exists, is hydrolyzed to a greater extent. It may be pointed out that the decrease in dielectric capacity of the solution consequent upon the rise in temperature may be an important factor. Whether denatured protein in the absence of salt could be flocculated even at room temperature with sensible velocity by a suitable alteration in dielectric capacity of the system does not appear to have been investigated experimentally.

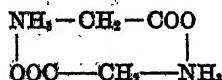
In addition to possible alteration in physical environment or in the chemical nature of the molecular individuals present, the main effect of increasing temperature will be of course the well-recognized one of increasing the intrinsic energy of the molecular species present. This aspect is taken up later in the present section in connection with the temperature coefficient of the flocculation process.

Apart from actual flocculation, however, as Chick and Martin have recorded, it is possible to get turbidity or opalescence even in the absence of salt on heating over a wide pH range from about 5 or 6 to about 2, so far as I can estimate it. From the point of view of stability of the protein solution, turbidity is of greater importance than complete flocculation since it indicates the initiation of aggregation. Further systematic work is required in this field.

It is a very remarkable fact at first sight that denatured protein molecules which contain amino and carboxyl groups, some of them probably in the zwitterion form, do not flocculate spontaneously and readily at ordinary temperature. It has to be remembered, however, as pointed out earlier, that to maintain a structure of the

type $^+NH_3 \cdot R \cdot COO^-$ it is necessary to assume that a considerable number of the lines of force associated with the two charged regions mutually form a closed field. If this were not the case one would naturally expect addition of H^+ and OH^- (from the solvent) with formation of the unionized form $NH_3 \cdot R \cdot COOH$. Consequently, although the zwitterion looks at first sight to be a highly reactive form capable of forming chains of molecules through the operation of simple electrostatic forces, the fact that this is not the case can only mean that the two electrified regions have to a large extent neutralized each other's field. The function of increase in temperature would therefore seem to be that of opening up the electrical fields around both the basic and acidic regions. On the other hand, the function of the neutral salt would seem to be essentially that of destroying the symmetry of the zwitterion formation, thereby opening up one portion of the molecular field, thus enabling it to react with the opposite field of the molecules which have not interacted with the salt.

As pointed out in the Appendix the simplest amino acid, glycine, which according to Bjerrum (69) exists almost entirely in the zwitterion form, is capable of exhibiting even in water a small but appreciable degree of polymerization. If the polymer has the structure $^+NH_3 \cdot CH_2 \cdot COO \cdot NH_3 \cdot CH_2 \cdot COO^-$ it is evident that such polymerization would be the first step towards still further union. The fact that the polymerization only goes as far as the formation of the double molecule in the case of this very simple type of amino acid rather suggests that here the most stable form is the "inert"



structure. Whatever the structure in the case of glycine, the polymerization is detectable only with solutions which are atleast 0.2 M. In the case of solutions of protein, the concentrations normally employed lie between 1 per cent and 10 percent, that is, for a protein of molecular weight 43,000, the concentration is only of the order 10^{-3} to 10^{-4} M. It is evident, therefore, that

appreciable polymerization even to double molecules is unlikely to occur, provided of course, that the process be regarded as reversible, as it is in the simple case of the amino acid. It is evident that the ions of the neutral salt play a definite rôle and, since small amounts suffice, the effect is not to be attributed to a general alteration in the properties of the solvent as a whole, but must be essentially a localized effect. Parenthetically, we may contrast this effect with the function of the salt (e.g., ammonium sulfate) in large concentration when acting as an agent for bringing about crystallization of undenatured protein. In the latter case the effect is almost certainly a "general" one in the main—namely, competition with the protein for solvent molecules. In Hopkins's words "the protein crystals are formed under the influence of the electrolyte but not in association with it."

Returning to the phenomenon of flocculation near the iso-electric point in the presence of inorganic salts, it has been observed in electrophoresis experiments that, in certain cases, part of the protein travels towards the anode and part towards the cathode, the remainder (usually a considerable portion of the whole) remaining motionless. This behavior indicates the existence of individuals such as $^+NH_3 \cdot R \cdot COONa$ and $Cl \cdot NH_3 \cdot R \cdot COO^-$, small quantities of which may likewise be inferred from the e.m.f. experiments of Pauli and the membrane measurements of Northrop and Kunitz already discussed in an earlier section. Such units, having lost their zwitterion character, and having the field of force which emanates from the group carrying the free charge thereby greatly enhanced, may now be capable of uniting with one of the original zwitterions themselves, thereby giving rise to a double molecule of the form



in which the group carrying the free charge has a similarly enhanced field, thereby rendering further addition possible. This probably represents the function of the neutral salt at the iso-electric point. Incidentally, if a very large amount of neutral salt be added, it is conceivable that, instead of getting the chain effect which is essential for flocculation, we might get simple fully

saturated complexes of the form $\text{Cl}\cdot\text{NH}_3\cdot\text{R}\cdot\text{COONa}$, which would be useless for flocculation and would possibly lead to increased stability of the protein.²⁰

If the above view of the function of the neutral salt as the accelerator of flocculation at the iso-electric point be correct—that is, if the flocculation or chain formation is caused not so much by neutralizing a free charge as by liberating the field of force around the charge which has *not* reacted with the neutral salt ion—it follows that the mechanism of flocculation of a protein is very different from that envisaged in, let us say, Smoluchowski's theory of coagulation of a hydrophobe colloid such as gold sol. The distinction is clearly not simply one dependent on great or small affinity for water, although this may play a secondary part. In the case of the proteins, flocculation is apparently brought about by the removal of the amphoteric character on the part of a small proportion of the protein units, these modified units being now characterized by the possession of an electric field of a single sign of enhanced intensity, which renders them capable of initiating chain formation. In the case of gold sol, the individuals to begin with are all of the one sign, and the effect of the electrolyte is to reduce the charge below a certain magnitude. For this reason a strong acid acts towards gold sol exactly as would other strong electrolytes. The behavior is very different in the case of proteins. We have now to consider the flocculation of protein in acid solution as distinct from flocculation at the iso-electric point.

Addition of strong acid or base alters the pH of the system and causes the zwitterion formation to disappear, the protein being now in the form of protein salt, e.g., protein chloride or sodium proteinate. H^+ ion and OH^- ion, in respect to their effect upon

²⁰ Actually it does not appear to be essential to postulate the production of definite chemical individuals of the type $^+\text{NH}_3\cdot\text{R}\cdot\text{COONa}$ or $\text{Cl}\cdot\text{NH}_3\cdot\text{R}\cdot\text{COO}^-$. Either of the ions of the neutral salt, if brought sufficiently close to the oppositely charged grouping of the zwitterion structure, would be expected to give rise to the effect without forming a constituent of the resulting coagulum. We might regard the function of the ion lying between two oppositely charged dipoles or zwitterion structures as that of locally lowering the dielectric capacity and thereby increasing the forces of attraction between the protein units.

protein, stand wholly apart from other ions in that their combination with protein is much more extensive and complete. For this reason the effect of adding acid is to give rise to protein units *all* similarly charged (cations) together with a greater or less proportion of unionized salt, e.g., protein chloride, the amount and composition of which is a function of the pH. The similarly charged protein ions repel one another and, so far as this portion of the protein material is concerned, the conditions for flocculation are even less favorable than they were prior to addition of the acid. On addition of neutral salt to the acid solution there is no doubt that union to some extent takes place between the anions of the salt and the protein cations in solution. Obviously this process could not conceivably lead to chain formation; in fact, addition of a very small amount of neutral salt would not be expected to have any appreciable influence. The only kind of effect to be anticipated would seem to be a mass action effect with alteration in solubility of the protein in the ordinary sense of the term. The idea that it is a mass action effect is not novel. It is substantially that put forward by Hardy in 1905 (70) in connection with the globulins.

Now it is evident that appreciable mass action effects cannot be brought about unless the neutral salt added increases appreciably the concentration of the anion already present by virtue of the acid added in the first place. That the effect of the addition of neutral salt under such conditions, i.e., at a pH well removed from the iso-electric point, is due to mass action, and that the process is one of precipitation rather than of flocculation, such as occurs on addition of salt *at* the iso-electric point, appears to be borne out by the experimental evidence available. This evidence may be illustrated by two quotations, one from the paper by Chick and Martin (71), the other from the paper of Hardy already referred to.

Chick and Martin state: "We found, however, that a solution of purified crystals of egg albumin which has been dialyzed until it is free of Am_2SO_4 does not form a precipitate on heating in the presence of HCl. Precipitation occurs, however, if neutral salts are added, but the amount of a chloride which is necessary to

effect agglutination makes the total chlorides to be estimated in the filtrate so great that the determination of any loss of chlorine (chloride ion) by fixation of HCl is rendered uncertain. A very small addition of a sulfate of sodium, potassium, or ammonium is able to bring about complete separation of the heated protein in a particulate form. In the presence of 0.014 *N* HCl and 0.36 per cent Na₂SO₄ we found that, although acid disappeared on coagulation, the whole or nearly the whole of the chlorine remained in the filtrate. On subsequent experimentation we ascertained, however, that a corresponding quantity of SO₄²⁻ had combined with the coagulum. We therefore had recourse to H₂SO₄ for acidifying the solution of egg albumin crystals, and a small quantity of Na₂SO₄ was added to facilitate separation of the coagulum. Only a small amount of this salt is required because SO₄²⁻ assists the agglutination of denatured egg albumin much more powerfully than Cl⁻. The slightly misleading phrase in the above is "very small" addition of a neutral salt, as this would naturally be taken to mean an addition that would not materially affect the concentration of the significant ion, in this case the anion. That the concentration is in fact very materially affected is shown by the analytical data given by Chick and Martin for the case just referred to. On analyzing the precipitate it is found that "the protein acid salt formed on addition of H₂SO₄ to a solution of pure crystalline egg albumin is precipitated as such on coagulation by hot water." The data show clearly that practically equivalent amounts of hydrogen ion and sulfate ion are removed. Furthermore, the precipitate contains in round numbers 1×10^{-3} equivalents of either H⁺ ion or SO₄²⁻ ion per gram of protein, indicating that the precipitate is in fact undissociated protein salt with practically all the available amino and carboxyl groups in combination with H⁺ ion and SO₄²⁻ ion. In the actual case considered the solution in equilibrium with the precipitate was distinctly acid, namely, $9.6 \times 10^{-5} N$. Had no sodium sulfate been added "to facilitate separation of the coagulum," this figure would likewise represent the concentration of SO₄²⁻ in equivalents. Actually, owing to the addition of Na₂SO₄, apparently in very small quantity (namely, to about 0.1 per cent) the concentration

of SO_4^{2-} is raised to $25 \times 10^{-3} N$, a very appreciable increase and one not unlikely to throw the protein sulfate out of solution, owing to the operation of the solubility product principle.²¹

A rather sharp distinction has been drawn between the kind of structure to be expected in the coagulum formed at the iso-electric point and the structure of the protein salt precipitated at a pH removed from the iso-electric point. In the latter case removal of the excess neutral inorganic salt by washing and addition of water should cause the precipitate to redissolve. In the former case such treatment should be ineffective, re-solution being brought about only by addition of acid or alkali. This is in general agreement with experiment. It is evident that much valuable information regarding the structure of the precipitate at different pH values might be obtained by systematic x-ray measurements. So far as the writer is aware no such measurements have as yet been carried out.

Supplementing the findings of Chick and Martin quoted above in connection with egg albumin, we now give a short quotation from Hardy in connection with (serum) globulin.

"Globulin itself is insoluble in water—. Acid globulin and alkali globulin can be separated in the solid state by dissolving globulin with minimal amount of acid or alkali and evaporating to dryness *in vacuo* over sulfuric acid and caustic potash. When hydrochloric acid is used, the dried HCl-globulin is found on analysis by Carius's method to contain all the chlorine used to dissolve the globulin. HCl-globulin therefore is stable *in vacuo* in the presence of solid KOH, and the acid may be regarded as being in true combination. Acid globulin dissolves in water."

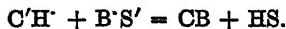
"Alkaline globulin can be separated in the solid state from, for example, solution in ammonia by drying *in vacuo* over sulfuric acid. When redissolved the solid shows the same order of molecular conductivity as the solution from which it was dried out. Both acid and alkali globulins ionize in solution."

"Solutions of acid or alkali globulin are precipitated by the addition of neutral salts; further addition brings about re-solution always in the

²¹ It follows that the precipitate formed under these conditions is not flocculation in the sense of molecular chain formation, such as appears to be the case at the iso-electric point.

case of alkali globulin, sometimes in the case of acid globulin. Still more salt brings about reprecipitation. In the first solution the globulin is ionic, in the second solution it is not ionic."

"Now if the action between salt and colloid is chemical and identical, for instance, with the selective reaction in dyeing which discriminates between acid and basic dyes, it would be represented by a generalized equation of the form:



That is to say, the colloid functions as an acid and by the law of mass action the compound CB is formed because of its insolubility."

As is generally realized, the behavior of globulins with respect to neutral salts is of a more complex character than the behavior of proteins of the albumin type. Until further information is available regarding the mechanism of flocculation and precipitation in the case of the latter, it will be readily understood that many features of the former are at the present time so obscure that speculation is valueless.

We may at this point draw a comparison between the mechanism of the precipitating action of a weak acid, such as acetic acid, to be anticipated on the basis of the considerations (neither novel nor original) which we have attempted in the foregoing to emphasize as at least offering a certain amount of guidance through a subject complex in itself and rendered more so by incomplete data. The two cases to be considered are (*a*) the precipitation of material of the albumin type and (*b*) the precipitation of a colloidal metal. In the case of the hydrophile colloid (albumin) the hydrogen ion and acetate ion would be expected to be equally involved in the precipitation, the hydrogen ion combining with the protein (initially iso-electric) to give rise to protein cations, which in the presence of a sufficiency of acetic acid unite with acetate ions to form protein acetate until, the solubility of the salt being exceeded, the protein would be expected to precipitate in the form of the salt.

In the case of the hydrophobe colloid (say gold sol) the hydrogen ion is primarily concerned, its rôle being that of diminishing or annulling the initial negative charge on the colloid particles,

which now aggregate as such (not as the acetate) in the manner envisaged in the theory of Smoluchowski. In the first case one would expect the precipitate to be crystalline; in the second case amorphous—or, more precisely, to have the same degree of lattice structure as the original sol particles themselves.

Critical increment of flocculation

This section may be concluded with a brief consideration of the influence of temperature upon the flocculation process. This has been investigated by Chick and Martin for diluted horse serum, dialyzed, and containing acetic acid, together with either sodium chloride or ammonium sulfate. As the behavior observed is similar in both cases we shall consider one of them only, namely, a diluted serum (3.3 per cent) containing 3.7 cc. of 0.1 N acetic acid in 100 cc. and NaCl at a concentration of 1 per cent. Apparently the solutions are milky to begin with. The flocculation rate is determined by noting the times taken for "visible particles" to appear. From the temperature coefficient data given I have calculated the apparent energy of activation or critical increment, E , of the process. The results are given in table 5. Chick and Martin remark upon the fact of a very high temperature coefficient at the lower temperature range which falls to a low value of normal magnitude at higher temperatures. This is shown more clearly by the variation in the E values, a "normal" limiting value of about 23,000 calories being eventually attained. This value presumably represents the energy required to activate or open up the valency field around at least one amino or carboxyl group to enable it to unite with a polar group of opposite sign. (Although the connection is a little remote, it may be mentioned that the energy required to break the peptide linkage —CO—NH— in the hydrolysis of this group has been found in the writer's laboratory to be 22,000 calories per gram-molar group (Escolme and Lewis (28)). That a critical increment of even "normal" dimensions is obtained anywhere for the flocculation process is a striking observation. It indicates clearly that the act of aggregation in the case of denatured protein is very different in nature from the aggregation of a colloid such as gold

hydrosol. In the latter case it has been shown in the writer's laboratory with some degree of precision (cf. Butler (72)), that when sufficient electrolyte has been added (and the quantity is small) to completely neutralize the charge on the colloid particles, the effect of temperature on the rate of flocculation is quantitatively accounted for by the alteration in viscosity of the medium (water). In such a case there is no true critical increment,²² all collisions being effective. In other words, while the aggregation of colloidal gold under the conditions named is satisfactorily taken account of by the Smoluchowski theory, in the case of protein the process is much more analogous to a bimolecular chemical reaction.

It will have been noticed that the material employed by Chick

TABLE 5
Critical increment, E, of flocculation

TEMPERATURE °C.	E IN CALORIES
44	110,000
46	67,000
49	83,000
56	37,300
66	29,100
76	22,400
86	24,400

and Martin in connection with the influence of temperature is not a pure protein, nor were the initial solutions transparent. Furthermore the pH was evidently not determined. We do not know therefore whether the limiting critical increment is a function of pH or not. It would be desirable to know with some degree of precision the critical increment of pure crystallized egg albumin with respect to flocculation after preliminary denaturation. The values for the rate of flocculation of egg albumin at different temperatures recorded by Lepeschkin (18) are too inaccurate to

²² Expressed as a fictitious critical increment, the viscosity effect amounts to 1000 calories (in round numbers) for a process occurring in an aqueous system in the neighborhood of 25°.

allow of any conclusion being drawn as to the magnitude of the increment or as to whether it varies with temperature or not.

The very great influence of temperature on the flocculation of horse serum at the lower temperature region is considered from a statistical standpoint by Chick and Martin. It would seem, however, that such statistical considerations applied to intrinsic energy relations for an individual process taken to be essentially the same in nature at different temperatures would be consistent with a constant value for the critical increment²³ and not with one rapidly altering as temperature rises.

The large value of E at the lower temperature range at first sight suggests that denaturation is occurring at that region and that in fact we are dealing not with a single process but with two consecutive processes. Chick and Martin, however, state that the material was denatured to start with. That such an explanation based on two processes could not explain the fall in E with rise in temperature may also be shown on theoretical grounds. Let us suppose that there are two processes $A \rightarrow B \rightarrow C$, in which $A \rightarrow B$ with a velocity constant k_1 is the slow process, and $B \rightarrow C$ with velocity constant k_2 is the faster process. On this basis the observed critical increment over any feasible temperature range should be the critical increment of the slower process and should not vary with temperature. It is equally useless to assume that

two simultaneous side processes $A \begin{smallmatrix} \nearrow B \\ \searrow C \end{smallmatrix}$ occur, as it may be shown

in this case that the critical increment observed should be sensibly that of the *faster* process and again would be expected to be independent of temperature. If we are to explain the variation in E on the basis of two processes it would seem to be necessary to assume the existence of *independent* processes of which the one with the higher critical increment peters out at a fairly low temperature. This would be a purely *ad hoc* assumption for which there is no evidence at all in the system examined,

²³ With $E > 20,000$ calories, statistical theory shows that over any temperature range at which a liquid system could exist as such, the fraction of molecules having the necessary critical energy is exceedingly small compared with the total. Consequently Chick and Martin's argument does not apply.

although the assumption of successive processes giving rise to breaks in the log k -temperature curve has been made in the much more complicated case investigated by Crozier and his co-workers (73) in connection with the chemical processes underlying certain vital activities in organisms. It would seem necessary therefore in the protein flocculation case, to revert to the concept of a single process and to attempt to account for the marked decrease in E as temperature rises by assuming that each protein unit itself undergoes some internal change—differing completely from denaturation, however—with increase in temperature, the change being of such a nature that the chemical groups—almost certainly the amino and carboxyl groups²⁴—at which flocculation may reasonably be regarded as initiating itself do not become more numerous but are possessed of a higher average energy content than is allowed for in ordinary statistical treatment. The suggestion amounts to the assumption that with increase in temperature there is not simply an all round increase in energy content distributed amongst a fixed number of degrees of freedom, but that in the protein unit a rise in temperature brings new degrees of freedom into operation. That is, a degree of freedom associated with some chemical bond may be in a quantized state at the lower temperature region with energy content small compared with that demanded on an equipartition basis, but on raising the temperature the energy content may increase towards the equipartition value. If now we regard the energy required to cause a given bimolecular chemical process to occur as being contributed partly by certain degrees of freedom within the chemical unit and partly by the kinetic energy of collision, as in the well-known theory of Hinshelwood and of Fowler (cf. Hinshelwood (74)), it follows that the assumption of a variable, as distinct from a fixed, number of degrees of freedom, whose energy is available for transfer in this sense, will

²⁴The fact that the precipitate can be dissolved by sufficient acid or alkali points to this. The attempt was made by Cubin (*loc. cit.*) in this laboratory to confirm this by using formaldehyde in the expectation that the flocculation would consequently be hindered. A slight hindering effect was observed but the results were not as conclusive as had been hoped.

modify the effect of temperature upon the chemical process and thereby conceivably give rise to an observed critical increment, which shall itself be a function of temperature and not independent thereof, as would be expected on the basis of a fixed number of degrees of freedom. I am indebted to Dr. A. McKeown of this Department for working out the consequences of this assumption. In brief, the assumption of an increase in effective²⁵ degrees of freedom with rise in temperature does in fact lead to a decrease in the observed or apparent critical increment with rise in temperature, and by inserting certain numerical values for the theoretical or true critical increment,²⁶ the number of effective degrees of freedom, and the rate of increase in degrees of freedom with temperature, an "apparent" critical increment altering with temperature in much the same way as that observed by Chick and Martin may be obtained. The real drawback to the treatment, possibly a fatal drawback, is the high value (> 0.1) which has to be ascribed to df/dT (the increase in the number of effective degrees of freedom per degree) in order to account for an apparent (or observed) critical increment, which can vary, as found by Chick and Martin, by as much as 50,000 calories for a rise of 40° in temperature.

No conclusion can therefore be drawn at the present time regarding the magnitude of the critical increment of flocculation and its apparently high variation with temperature. The problem, however, seemed to be of such importance as to justify a certain amount of space being given to it in the present connection.

²⁵ The effective degrees of freedom (f) are to be distinguished from the real internal degrees of freedom (g), the energy of which is quantized. The number, f , is in fact defined by the relation

$$f RT = g E(\nu_1 T)$$

where on the simplest basis

$$E(\nu_1 T) = \frac{\hbar\nu}{e^{\hbar\nu/kT} - 1}$$

In general f will be small while g would necessarily be considerably greater.

²⁶ This may with some confidence be taken as being in the neighborhood of 20,000 calories. It is in fact not far from the limiting value obtained by Chick and Martin at the high temperature region which they regard as the true value for the flocculation process.

APPENDIX

Note on the physicochemical behavior of glycine in solution

On the zwitterion theory, glycine exists in solution at its iso-electric point ($\text{pH} = 6.08$) mainly in the form $^+ \text{NH}_3 \cdot \text{CH}_2 \cdot \text{COO}^-$. In aqueous solution it is conceivable that electrically charged individuals of this kind, although not "free" ions in the ordinary sense, could exert a mutual influence analogous to that taken account of by the Debye-Hückel theory of interionic attraction. To test whether any such effect is operative a number of determinations of the lowering of the freezing point were carried out by Mr. W. J. Loughlin in the writer's laboratory. If Δ is the ob-

TABLE 6
Glycine in water

GRAM-MOLES OF GLYCINE ($M = 76$) IN 1000 GRAMS OF WATER	APPARENT MOLECULAR WEIGHT	$f_0 = \frac{\Delta}{\Delta_0}$	$x = 1 - \frac{\Delta}{\Delta_0}$	$K = \frac{(\text{GLYCINE})^2}{(\text{DIGLYCINE})}$
0.210	75.35	0.995	0.005	[41.2]
0.385	78.05	0.961	0.039	8.4
0.572	79.09	0.948	0.052	8.8
0.765	80.37	0.933	0.067	8.6
0.986	81.92	0.916	0.084	8.1
1.674	85.28	0.880	0.120	8.1
2.176	86.58	0.866	0.134	8.7
Mean value = 8.4				

served lowering of freezing point, and Δ_0 the theoretical lowering on the basis of a molecular weight of 75, then $\frac{75\Delta_0}{\text{apparent molecular weight}}$ represents the apparent molecular weight. Furthermore the ratio Δ/Δ_0 is the osmotic coefficient f_0 , as defined by Bjerrum. The values obtained are given in table 6.

On plotting the values of $(1 - f_0)$ against the concentration, a curve was obtained which differed significantly from that given by KCl. Thus with KCl the values of $(1 - f_0)$ rise rapidly at first and then much more slowly with increase in concentration of the salt. This change is taken account of in a reasonably satisfactory way by the Debye-Hückel interionic attraction theory, in which

allowance is made for the mean effective diameter of the ions in solution and for the thickness of the interionic attraction sphere. With glycine, on the other hand, the values of $(1 - f_0)$ at first remain low, then rise, passing through a point of inflexion with increase in content of glycine. It seems reasonable to conclude, therefore, that these osmotic abnormalities are not to be ascribed to forces of the "interionic attraction" type. It would seem justifiable to ascribe the increase in apparent molecular weight to actual polymerization, for which a zwitterion structure would be expected, on general grounds, to be eminently suited. On this basis, if x denotes the fraction of each mole of glycine polymerized to the diglycine form, then

$$\frac{\Delta_0}{\Delta} = \frac{1}{1-x}$$

and at a concentration c the mass action expression becomes

$$K = \frac{c(1-2x)^2}{x}$$

This quantity is given in the final column of table 6. In view of the constancy of K there seems little doubt but that polymerization does in fact occur. Thus a 1 M solution is polymerized to the extent of 10 per cent in round numbers. The existence of this polymerization has of course to be allowed for in any measurements upon the behavior of glycine in solutions of salts before one can assert with certainty that union of glycine with the salt occurs in *solution*.

Although the glycine zwitterions do not exert a detectable interionic attraction upon one another—in the sense of the Debye-Hückel theory—it was of interest to investigate the possible influence of a simple electrolyte, sodium chloride, upon the behavior of the glycine individuals. To this end a number of determinations of the lowering of the freezing point of solutions of glycine in 0.1, 0.2, and 1.0 N NaCl has been carried out in the writer's laboratory by Mr. A. M. Maiden. Selecting two concentrations of glycine—namely, 0.38 and 0.76 M (M being taken as 75 for the purpose of expressing molarity), the following results

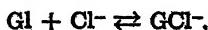
were obtained. The values obtained with pure water as solvent are taken from table 6. It will be observed that there is an increased osmotic abnormality caused by the presence of the NaCl and expressed in the form of an apparent molecular weight. The osmotic coefficient of Bjerrum is given in the final column, Δ_0 being replaced by Δ to allow for the fact that even apart from NaCl there is an osmotic abnormality (due to polymerization). Before attempting even a partial analysis of the above figures it is necessary to consider the evidence existing for chemical union between one or both of the ions of NaCl with glycine.

TABLE 7
Glycine in NaCl solutions

CONCENTRATION OF NaCl	APPARENT MOLECULAR WEIGHT	$\Delta'/\Delta = f_0$
Concentration of glycine = 0.38 M ($M = 75$)		
0	78.05	1
0.1	79.5	0.98
0.2	87.2	0.90
1.0	94.5	0.88
Concentration of glycine = 0.76 M ($M = 75$)		
0	80.4	1
0.1	82	0.98
0.2	88.7	0.91
1.0	95	0.85

Oryng and Pauli (75) have measured by the e.m.f. method—using the calomel electrode in the presence of glycine—the influence of glycine upon the activity of the Cl⁻ ion in KCl. Naturally the experiment gives no indication as to whether any union occurs between K⁺ ion and glycine. Employing the data of Oryng and Pauli, and introducing the data for the activity coefficient of Cl⁻ ion, ($\alpha_{Cl^-} = \alpha_{K^+}$) in KCl, as given in Lewis and Randall's "Thermodynamics," we may calculate the concentration of Cl⁻ in equilibrium with the glycine. The difference between this "free" Cl⁻ ion and that obtaining initially in the KCl solution employed (assuming complete ionization) will be taken as representing the amount of Cl⁻ combined with the

glycine. It is convenient to denote glycine by the symbol Gl. It is evident from table 8 that the e.m.f. data, particularly at the higher range of concentrations of KCl, are not sufficiently reliable to permit of any precise calculation. Nevertheless an attempt will be made to get at least an approximate idea of the magnitude of K_1 , the equilibrium constant of the process



in order to estimate the order of the combination effect upon the

TABLE 8
Influence of glycine upon the activity of the Cl⁻ ion in KCl
1.6 per cent glycine = 0.2133 M

INITIAL CONCENTRATION OF KCl	ACTIVITY OF Cl ⁻ ION IN (KCl + Gl) AS OBSERVED BY OYNG AND PAULI	CONCENTRATION OF FREE Cl ⁻ ION	CONCENTRATION OF BOUND Cl ⁻ = CONCENTRATION OF GCl ⁻
<i>N</i>			
0.05	0.04346	0.051	(negative)
0.02	0.01760	0.0194	0.0006
0.01	0.00882	0.0095	0.0005
0.005	0.00413	0.0044	0.0006
0.002	0.00151	0.00155	0.00045
0.001	0.00071	0.000724	0.00028

freezing point data obtained by Maiden for (greater) concentrations of NaCl. Strictly, K_1 is defined by

$$K_1 = \frac{\alpha_{\text{GCl}^-}}{\alpha_{\text{Gl}} \times \alpha_{\text{Cl}^-}}$$

We shall assume that the activity coefficient of glycine itself is identical with that of the glycine-Cl⁻ complex. That is,

$$K_1 = \frac{\alpha_{\text{GCl}^-}}{\alpha_{\text{Gl}} \times \alpha_{\text{Cl}^-}}$$

The values thus obtained are given in table 9. It may be pointed out that the values of the equilibrium constant of the process calculated on the basis of allowing for union with metallic cation simultaneously with the Cl⁻ ion, would necessarily show still greater increase with dilution of KCl.

With higher concentrations of KCl, *viz.* 0.1, 0.2 and 1.0 *N*, it is evident that the value of K_1 would probably be considerably lower than 0.1. Taking this as a maximum value, however, we may make an attempt to calculate a maximum value for the degree of union of glycine under these conditions. With glycine at an initial concentration of 0.38 *M*, we thus find that in presence of 0.1, 0.2, and 1.0 *N* KCl the concentration of the resulting GICl^- is 0.003, 0.0056, and 0.023 *M*. That is, the percentage of glycine in the form of the complex is 0.8 per cent, 1.5 per cent and 6 per cent respectively. In so far as glycine unites with one of the ions of KCl in a solution of this salt, the depression of freezing point caused by the glycine is thus less than it should be, and a correction factor has to be introduced to allow for that portion of the glycine which has been rendered osmotically in-

TABLE 9
Values of the equilibrium constant at different concentrations of KCl

	INITIAL CONCENTRATION OF KCl					
	0.05 <i>N</i>	0.02 <i>N</i>	0.01 <i>N</i>	0.005 <i>N</i>	0.002 <i>N</i>	0.001 <i>N</i>
K_1	—	0.16	0.266	0.68	1.4	1.9

active. This correction corresponds to the percentages of the total glycine concentration (0.38 *M*) already calculated as combined with chloride ion in 0.1, 0.2, and 1.0 *N* KCl solution, respectively. This would lead us to expect the values 0.99, 0.98 and 0.94, respectively, for Δ'/Δ in table 7 in place of the observed values 0.98, 0.91 and 0.84. Evidently the osmotic abnormality shown in table 7 (*i.e.*, the increase in apparent polymerization due to the presence of sodium chloride) can be ascribed only to a limited extent to union of glycine with Cl⁻ ion as calculated from the somewhat unsatisfactory data of Oryng and Pauli. In fact, if the total observed "excess" abnormality as expressed by the apparent molecular weight 94.5 for glycine in 1.0 *N* NaCl, were to be ascribed entirely to combination of a certain fraction of the glycine with Cl⁻ ion, it would be necessary to conclude that 0.066 moles of glycine were thus

combined out of a total of 0.38 moles; in other words, that the degree of union was 17 per cent. While this is, of course, not impossible, it is far greater than anything suggested by the results of Oryng and Pauli,

The excess osmotic abnormality due to the presence of NaCl could conceivably be ascribed, not to true polymerization nor to chemical union with Cl⁻ ion, but to an interionic attraction effect in the sense of Debye and Hückel, exerted by the ions of the salt upon the zwitterions of glycine. This possibility has now to be considered.

Treating the excess osmotic abnormality in terms of the Debye-Hückel theory, it is necessary in the first place to convert the osmotic coefficients f_0 for glycine recorded in table 7 into the corresponding activity coefficients (α). Since we have shown that the osmotic coefficients are sensibly the same for 0.38 M and 0.76 M glycine, in the presence of sodium chloride, we shall take the mean values of these as a starting point and assume that the α values in this case are related to f_0 values in the same way in which they are related in the case of ionized KCl.²⁷ This is naturally an assumption based solely upon the univalence of the charges in the two cases compared. In this way one obtains the values of α and of $-\log \alpha$ for glycine, recorded in table 10 as "obtained from the freezing point data." To compare this result with that of the Debye-Hückel theory we shall employ the expression

$$-\log_{10} \alpha = \frac{z^2 \times 0.5 \sqrt{\sum c \nu^2}}{1 + 1.5 \sqrt{\sum c \nu^2}}$$

See Cohn and Prentiss (53).

In this expression α stands for the activity coefficient of the glycine zwitterion of valency z in the presence of NaCl, the concentration of either Na⁺ or Cl⁻ being c , and ν , the valency thereof, being unity. Setting $z = 1$ it follows that

$$-\log_{10} \alpha = \frac{0.5 \sqrt{2c}}{1 + 1.5 \sqrt{2c}}$$

²⁷ For this case at the fairly high concentration range here considered the purely empirical relation $f_0 = \sqrt{\alpha}$ was used for purposes of computation. The same relation holds very approximately for cane sugar in solution.

The values of $-\log_{10}\alpha$ are likewise included in table 10. From the degree of agreement exhibited between "calculated" and "observed" values of $-\log_{10}\alpha$, it is practically impossible to decide whether interionic action plays any appreciable part or not.²⁸ If the value of $-\log_{10}\alpha$ obtained for glycine at the lower concentration of salt be regarded as definitely disproving the applicability of interionic attraction, we would be forced to regard the apparently high molecular weights of glycine in the presence of salt as indicating, in fact, increased polymerization. No decision on this important question is possible until further experimental data are available, especially in the region of low salt content.

TABLE 10
Activity coefficients of glycine in the presence of NaCl

CONCENTRATION OF NaCl	MEAN OSMOTIC COEFFICIENT, f_0 , OF GLYCINE	MEAN VALUE OF α "FROM FREEZING POINT DATA"	$-\log_{10}\alpha$ (FROM PREVIOUS COLUMN)	$-\log_{10}\alpha$ (DEBYE AND HÜCKEL THEORY)
0	1	1	0	0
0.1	0.98	0.94	0.024	0.134
0.2	0.91	0.75	0.123	0.162
1.0	0.84	0.59	0.227	0.230

Similar inconclusiveness exists in connection with the attempt to calculate the influence of salts upon the solubility of glycine. Taking Pfeiffer's data (42), which refer to salt concentrations of the order of 0.5 to 1.0 M, we find that $\log_{10} \frac{S}{S_0} = 0.0342$, where S_0 stands for the solubility of glycine in water and S for its solubility in 0.97 M LiCl. On the theory of interionic action we would expect this quantity to be very much greater—namely, 0.225. Similarly for 0.57 M CaCl₂, the observed value of

²⁸ If allowance were made in column 2 of table 10 for the fraction of glycine combined with Cl⁻, the mean osmotic coefficients for the free glycine would not have fallen so rapidly as the values of column 2 indicate. The corresponding α values obtained therefrom would likewise not fall so rapidly and hence the value $-\log_{10}\alpha$ obtained from them, in turn would rise less rapidly than the values given in column 4. This would mean that the discrepancies between columns 4 and 5 would be slightly greater than they appear to be in the table.

$\log_{10} \frac{S}{S_0}$ is 0.092, the calculated value being 0.245. In neither case has any allowance been made for union of Cl^- ion with the glycine. Also no allowance has been made for the change in dielectric capacity of the saturated solution which, owing to the enormous solubility of glycine in water, may well be considerable, in view of Fürth's observation (76) that glycine solutions possess a greater dielectric capacity than water itself. Thus, with a glycine content of 16.6 weight per cent, the dielectric capacity D attains an apparently asymptotic value of 93.0 instead of 80.5, the value taken by Fürth for pure water. The factor 0.5, which appears in the numerator of the Debye-Hückel expression already employed, contains $1/D^{\frac{1}{2}}$. The denominator term 1.5 contains $1/D^{\frac{1}{2}}$. Neglecting the effect of the latter, it follows that the calculated value of $\log_{10} \frac{S}{S_0}$ would become 0.180, instead of 0.225, for a solution of glycine containing 0.97 M LiCl. This is still vastly greater than the observed value 0.0342.

Finally, it is of interest to see what effect is produced upon the osmotic coefficient of glycine when sucrose is added to the solution, especially in view of the fact that sucrose diminishes the dielectric capacity. Thus Fürth (in agreement with others) finds that the dielectric capacity of molar sucrose solution is 63 (water = 80.5). Using a 1 M solution of sucrose as the solvent, Maiden, in the writer's laboratory, has measured the depression of freezing point produced by the addition of different amounts of glycine. The volume concentrations of glycine varied between 0.23 and 0.96 M . In computing the actual mass concentrations, it is necessary to refer the mass of glycine to a certain mass of water. An ambiguity arises as to whether or not allowance should be made for the water of hydration of sucrose (6 moles of water per mole of sucrose). On general grounds one would expect that the water bound by the sucrose would be unavailable as solvent for the glycine. The results of both methods of computation are given in table 11. A further point arises as to the correct value to assume for the theoretical molecular depression A_m . For water this is taken as 1.86° . For molar sucrose the

value obtained by plotting the molecular depression of sucrose against concentration in solutions of sucrose alone in water is 2.103°. Results are recorded on the basis of both.

Table 11 contains sets of values for the apparent molecular weight. The second column gives the value of M calculated on the basis $\Delta_0 = 1.86$, the total water in the solution being regarded as solvent; similarly for the fourth column. The third column gives the value of M on the basis $\Delta_0 = 1.86$, the effective solvent being total water less the amount bound by the sugar; similarly for the fifth column. The point is whether these relatively high values for M in the presence of sucrose represent real polymerization in excess of that in water alone (shown in the last column of

TABLE 11
Apparent molecular weight of glycine in 1.0 M sucrose solution

GLYCINE, VOLUME CONCEN- TRATION, MOLES/LITER OF SOLUTION	M CALCULATED ON THE BASIS OF $\Delta_0 = 1.86$	M CALCULATED ON THE BASIS OF $\Delta_0 = 1.86$	M CALCULATED ON THE BASIS OF $\Delta_0 = 2.103$	M CALCULATED ON THE BASIS OF $\Delta_0 = 2.103$	M OF GLYCINE IN WATER
	W_{Total}	W_{Free}	W_{Total}	W_{Free}	
0.233	82.4	95.6	93.4	108	75.8
0.470	82.5	95.7	93.5	108	78.5
0.483	82.9	96.2	94.0	109	78.7
0.70	84.9	98.6	96.2	111	80.1
0.72	84.3	97.3	95.5	111	80.2
0.96	84.6	98.2	95.9	111	81.9

table 11), an effect which would, on general grounds, be favored by the decreased dielectric constant, or whether, quite apart from polymerization, the activity of the glycine has been diminished by combination with sucrose or otherwise to the extent represented by the figures. We shall consider first the question of increased polymerization of glycine due to the presence of sucrose. The most direct way of testing this would be by calculating the equilibrium constant for the assumed polymerization process. Denoting the (new) equilibrium constant by K' (to distinguish it from K , the value of the constant obtained for glycine in water alone) we may write

$$K' = (1 - \frac{2}{M})^2$$

The quantity x is given by $(M - M_0)/M$, where M is the apparent molecular weight. c is taken as the number of moles ($M_0 = 75$) of glycine per liter of solution. Naturally the values of K' differ according to the basis of calculation employed for M , as shown in table 11. On calculating the values of K' it is found in all cases that K' apparently increases approximately three-fold as the volume concentration of glycine is increased from 0.233 to 0.96 moles per liter. For this reason it is not thought worth while to record the actual values obtained for K' . It follows that the concentration of diglycine does not increase sufficiently rapidly with increase in total glycine. In other words, to obtain a constant value for K' the apparent molecular weights should have increased with concentration more rapidly than they have been observed to do. Incidentally, the assumption of the union of an appreciable amount of the glycine with sucrose (considered below), could not account for the observed molecular weight being too low, since obviously any such union would tend to give fictitiously large molecular weights. It would seem, therefore, that the conclusion to be drawn is that the osmotic abnormality exhibited by glycine in the presence of sucrose is not to be attributed to further polymerization. We are left therefore with the alternative—namely, that the activity coefficient of glycine may be diminished owing to the presence of the sucrose. It would be natural to ascribe this in the first place to union of sucrose with glycine.

On this basis the extent of union has been calculated from the data of table 11, postulating the simplest type of complex—namely, one molecule of glycine with one molecule of sucrose. The corresponding dissociation constants of the complex have likewise been calculated. Naturally different extents of union are obtained according to the column of table 11 chosen. In all four series, however, roughly constant values for the dissociation constant are obtained, the values obtained on the basis of $\Delta_0 = 1.86$, W_{Total} being the least satisfactory. No distinction can be drawn as between the columns 3, 4, or 5 of table 11. A more extensive series of measurements would be required. It may be of interest to record the values thus obtained for the extent of

association of glycine with sucrose when 0.5 mole of glycine is present in a 1.0 *M* sucrose solution. The degrees of association amount to 5 per cent, 18 per cent, 16 per cent and 27 per cent according as the data of columns 2, 3, 4, or 5 of table 11 are employed for the calculation. None of these values is inherently improbable.

Mr. W. J. Shutt of this Department has determined the solubility of glycine in water and in 1.0 *M* sucrose, respectively, at 0°C. The analytical method involved formol titration of the weighed sample, using 0.1727 *N* NaOH, and phenolphthalein. The solubility in water was found to be 14.20 grams of glycine per 100 grams of water. In the presence of 1.0 *M* sucrose the solubility²⁹ was found to be 9.90 grams per 100 grams of sugar + water. Since the density of the sugar solution is 1.131, the solubility may be expressed as 14.19 grams per 100 grams of total water. If all the water present were regarded as available solvent, one would conclude that the sucrose has no effect at all upon the activity coefficient of glycine. On the more probable assumption that only part of the water is available, it follows that there is a real increase in solubility, that is, a decrease in the activity coefficient of glycine, due to the sucrose either as an environmental effect or due to complex formation. This may be expressed quantitatively on the basis that the sucrose holds 6 molecules of water per molecule of sugar, this quantity of water being unavailable as solvent. We thus calculate that the solubility of glycine, in the presence of 1.0 *M* sucrose, is 16.4 grams per 100 grams of "free" water. Hence the activity coefficient, α , of glycine in the presence of the sucrose, relative to its value in water, is 14.2/16.4 or 0.866. Assuming that this value would hold for any concentration of glycine in the presence of sucrose, and employing the empirical relation $f_0 = \sqrt{\alpha}$ we obtain for f_0 , the osmotic coefficient of glycine in the presence of molar sucrose, the value 0.954. The apparent molecular weight in the presence of sucrose would therefore be expected to be the observed value in water (corresponding to the concentration of glycine employed) divided by f_0 . Hence for concentrations 0.470 and 0.72 *M*,

²⁹ The solid phase was pure glycine.

respectively, the apparent molecular weights should be 82.3 and 84.1. (The corresponding values in water are 78.5 and 80.2.) Although these are close to the results given in the first column of table 11, it would be unsafe and indeed illogical, to assume that this column rather than the remaining ones was to be preferred, in view of the approximate nature of the empirical relation used to connect α with f_0 , and also in view of the assumption regarding the applicability of the α value, obtained from solubility data, to the region of more dilute glycine solutions. Owing to the absence of data it is impossible at the present time to carry out a more satisfactory analysis of the osmotic behavior of glycine in the presence of sucrose.

The behavior of glycine in solution outlined in this Appendix, and the general inconclusiveness which exists at the present time regarding many aspects of its behavior may at least serve to indicate how much still remains to be done before a quantitative treatment of the behavior of protein solutions in terms of activity and interionic attraction can be effected.

Addendum

Since the above account was written a paper by M. Frankel (77) has appeared upon molecular weight determinations (boiling point as well as freezing point) for a number of amino acids in water,—in the absence of salts. Frankel finds that association is exhibited by glycine, *l*-asparagine, *d*-arginine, and *d*-dioxyphenylalanine. On the other hand, *d*-, *l*-, and *dl*-alanine have a normal molecular weight. In the case of glycine, the most concentrated solution employed contained 1.809 moles per 1000 grams of water. At this concentration the molecular weight was found by Frankel to be 88.3, which is somewhat higher than the value obtained in the writer's laboratory—namely, 85.3 at a concentration of 1.674 moles per 1000 grams of water, and 86.6 at a concentration of 2.176 moles. More recently still a paper by Borsook and MacFadyen (78) has appeared dealing with "The effect of iso-electric amino acids on the pH of a phosphate buffer solution," in which the relative merits of the "classical" and zwitterion conceptions of amino acids are discussed. The importance of

dielectric capacity measurements is pointed out, as has likewise been done independently in the present paper.

Summary of appendix

1. From freezing point data it is shown that glycine in water is polymerized, to the extent of 10 per cent (in round numbers) in a 1.0 M solution. Apparently the glycine zwitterions do not exert an appreciable "interionic attraction" upon one another.

2. In the presence of sodium chloride, there is an increased osmotic abnormality. It is shown that the extent of union of glycine with chloride ion is probably too small to account for more than a part of this abnormality. Evidence is also afforded that the effect is not due to increased polymerization. On ascribing the effect to interionic attraction the results obtained are inconclusive, especially in the region of low sodium chloride content.

3. In the presence of sucrose the freezing point data indicate osmotic abnormality in the sense of an increase in the apparent molecular weight. From an analysis of the results it is concluded that further polymerization does not occur, but that the effect is to be referred to a decrease in the activity coefficient of glycine due to the presence of the sucrose and probably due to complex formation. This is confirmed by solubility determinations in so far as this procedure is justifiable.

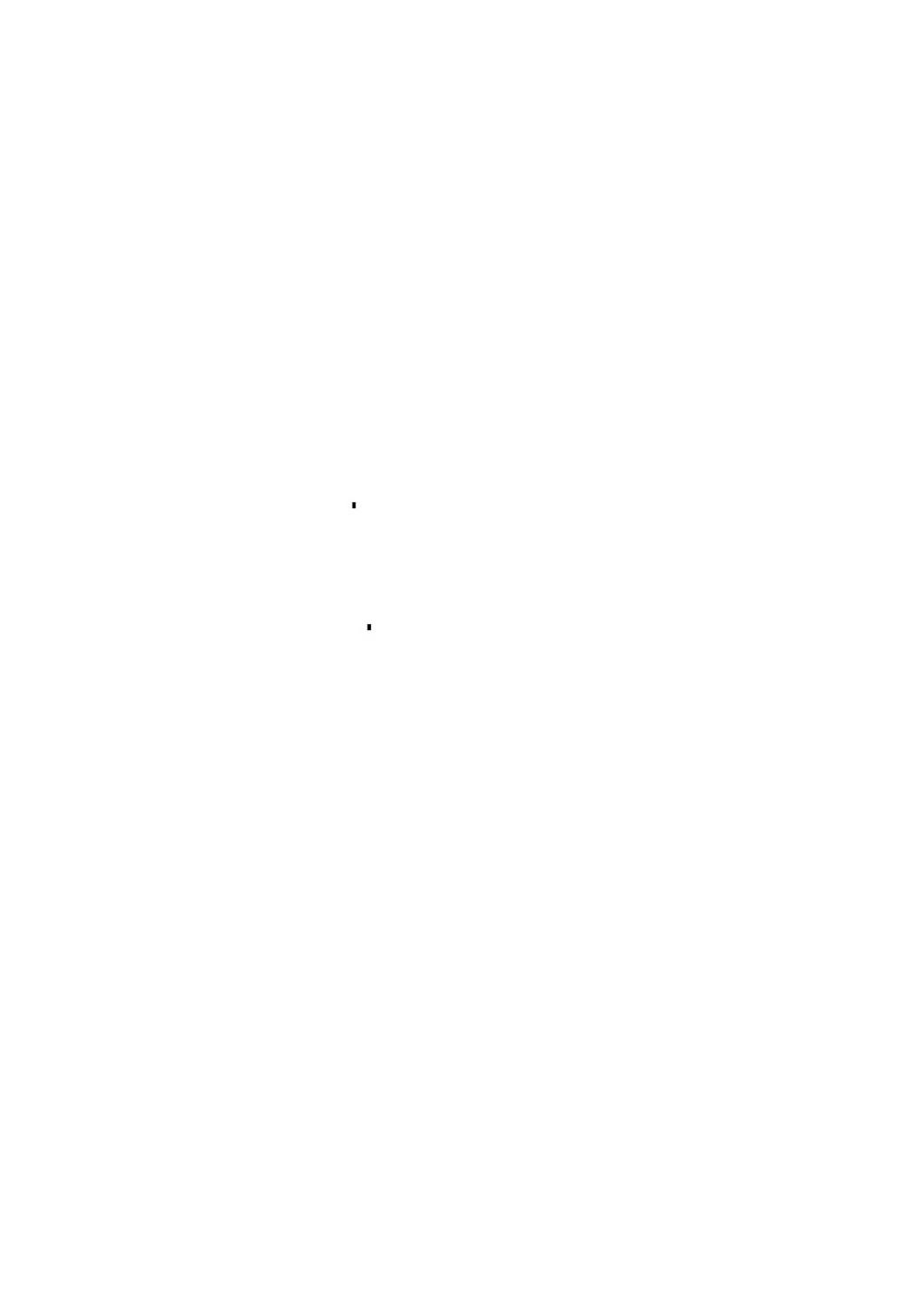
In conclusion the writer wishes to express his very great indebtedness to his colleagues Dr. R. O. Griffith, Mr. W. J. Shutt, and Dr. A. McKeown for their helpful criticisms and suggestions in connection with a number of the points considered and discussed in the above article.

REFERENCES

- (1) BOOTH: Biochem. J. 24, 158 (1930).
- (2) WILLIAMS: J. Am. Chem. Soc. 50, 2850 (1928).
- (3) FOWLER: Statistical Mechanics, p. 314. Cambridge University Press, London (1929).
- (4) CHICK AND MARTIN: Biochem. J. 7, 380 (1913).
- (5) SØRENSEN: Compt. rend. trav. lab. Carlsberg 12, 184 (1910).
- (6) SØRENSEN AND PALMSON: Compt. rend. trav. lab. Carlsberg 15, No. 2 (1923).
- (7) PREYER: Die Blutkrystalle, Jena (1871), quoted by H. G. Robertson in The Physical Chemistry of the Proteins, p. 49. Longmans, Green and Co., New York and London (1918).

- (8) MARRACK AND HEWITT: Biochem. J. 23, 1076 (1929).
(9) MATHEWS: Physiological Chemistry. W. Wood and Co., New York (1923).
(10) PALMER AND RICHARDSON: Colloid Symposium Monographs, Vol. III, p. 112. Chemical Catalog Co., New York (1925).
(11) CHICK AND MARTIN: J. Physiol. 40, 404 (1910); 43, 1 (1911).
(12) LEWIS: Biochem. J. 20, 965, 978, 984 (1926); 21, 46 (1927).
(13) CUBIN: Biochem. J. 23, 25 (1929).
(14) CHICK: Biochem. J. 8, 261, 404 (1914).
(15) TALARICO: Compt. rend. soc. biol. 68, 662 (1910).
(16) LIN, WU AND CHEN: Chinese J. Physiol. 2, 107-130 (1928).
(17) LINSSIER: Compt. rend. soc. biol. 68, 709 (1910).
(18) LEPESCHKIN: Biochem. J. 16, 678 (1922).
(19) ANSON AND MIRSKY: J. Gen. Physiol. 13, 121, 133 (1929); ibid. 13, 469, 477 (1930).
(20) MOELWYN-HUGHES: Trans. Faraday Soc. 25, 81, 503 (1929).
(21) WILLEHEIM: Kolloid-Z. 48, 217 (1929).
(22) BUGARSKY AND LIEBERMANN: Arch. ges. Physiol. 72, 51 (1898).
(23) MANABE AND MATULA: Biochem. Z. 52, 369 (1913).
(24) ROHONYI: Biochem. Z. 44, 165 (1912).
(25) CHICK AND MARTIN: J. Physiol. 43, 1 (1911).
(26) NORTHRUP AND KUNITZ: J. Gen. Physiol. 7, 25 (1925).
(27) HOFFMAN AND GORTNER: Colloid Symposium Monographs, Vol. II, p. 209. Chemical Catalog Co., New York (1925). GORTNER: J. Biol. Chem. 74, 409 (1927).
(28) ESCOLME AND LEWIS: Trans. Faraday Soc. 23, 651 (1927).
(29) ORYNG AND PAULI: Biochem. Z. 70, 368 (1915).
(30) HITCHCOCK: J. Gen. Physiol. 5, 883 (1923).
(31) FRISH, PAULI AND VALKO: Biochem. Z. 164, 401 (1925).
(32) PAULI AND WIT: Biochem Z. 174, 308 (1926).
(33) SIMMIS: J. Gen. Physiol. 11, 613 (1928).
(34) COHN AND PRENTISS: J. Gen. Physiol. 8, 618 (1928).
(35) LOEB: Proteins and the Theory of Colloidal Behavior, p. 49. McGraw-Hill Book Co., New York (1922).
(36) SVEDBERG AND TISELIUS: J. Am. Chem. Soc. 48, 2272 (1926).
(37) WRIGHT: Biochem. J. 23, 352 (1929).
(38) KENDALL: J. Am. Chem. Soc. 38, 1480 (1916).
(39) PAULI AND MATULA: Biochem. Z. 80, 187 (1917).
(40) PAULI AND SCHON: Biochem. Z. 153, 253 (1924).
(41) NORTHRUP AND KUNITZ: J. Gen. Physiol. 11, 481 (1928).
(42) PFEIFFER: Organische Molekulverbindungen (Chemie in Einzeldarstellungen, Vol. 11, herausg. von J. Schmidt, Enke, Stuttgart (1922)).
(43) NORTHRUP AND KUNITZ: J. Gen. Physiol. 7, 25 (1925); 9, 351 (1926); 11, 481 (1928).
(44) HITCHCOCK: J. Gen. Physiol. 6, 95 (1923-24).
(45) SIMMIS: J. Gen. Physiol. 11, 629 (1927-28).
(46) IPO AND PAULI: Biochem. Z. 213, 95 (1929).
(47) CHICK AND MARTIN: J. Physiol. 43, 1 (1911); 45, 261 (1912).
(48) THOMAS AND NORRIS: J. Am. Chem. Soc. 47, 501 (1925).

- (49) HARDY: J. Physiol. 33, 251 (1905); Proc. Roy. Soc. (London) 79B, 413 (1907).
(50) MELLANBY: J. Physiol. 33, 338 (1905).
(51) SØRENSEN: J. Am. Chem. Soc. 47, 457 (1925).
(52) OSBORNE AND HARRIS: Am. J. Physiol. 14, 151 (1905).
(53) COHN AND PRENTISS: J. Gen. Physiol. 8, 619 (1928).
(54) HARDY: J. Physiol. 33, 311 (1905).
(55) GORTNER, HOFFMAN AND SINCLAIR: Colloid Symposium Monographs, Vol. V, p. 192. Chemical Catalog Co., New York (1928).
(56) BANCROFT: Colloid Symposium Monographs, Vol. IV, p. 29. Chemical Catalog Co., New York (1926).
(57) COHN: Physiol. Reviews 5, 408 (1925).
(58) BJERRUM: Z. physik. Chem. 104, 168 (1923).
(59) CARMAN AND SCHMIDT: Phys. Rev. 30, 922, 925 (1927).
(60) MARINESCO: Compt. rend. 187, 718 (1929).
(61) MATHEWS: Am. J. Physiol. 14, 203 (1905).
(62) BJERRUM AND LARSSON: Z. physik. Chem. 127, 358 (1927).
(63) FÜRTH: Ann. Physik. 70, 63 (1923); cf. also SCHMIDT: *loc. cit.*
(64) RAMSDEN: J. Physiol. 28, xxiii (1902).
(65) SPIRO: Z. physiol. Chem. 30, 182 (1900).
(66) SCHMIDT: Phys. Rev. 30, 925 (1927).
(67) ARONSTEIN: Pflugers Arch. 8, 75 (1874).
(68) HEYNSTIUS: *ibid.* 9, 514 (1875).
(69) BJERRUM: Z. physik. Chem. 104, 147 (1923).
(70) HARDY: J. Physiol. 33, 281 (1905).
(71) CHICK AND MARTIN: J. Physiol. 43, 14 (1911).
(72) BUTLER: J. Phys. Chem. 34, 656 (1930).
(73) CROZIER AND CO-WORKERS: J. Gen. Physiol., Vol. 6, (1924) and later volumes,
especially Vol. 7, 189 (1925).
(74) HINSHELWOOD: Kinetics of Chemical Change in Gaseous Systems, 2nd
Edition. Oxford University Press, London (1929).
(75) ORYNG AND PAULI: Biochem. Z. 70, 388 (1915).
(76) FÜRTH: Ann. Physik. 70, 63 (1923).
(77) FRANKEL: Biochem. Z. 217, 378 (1930).
(78) BOOSOK AND MACFADYEN: J. Gen. Physiol. 13, 509 (1930).



A SYMPOSIUM ON NON-AQUEOUS SOLUTIONS¹

INTRODUCTION

W. V. EVANS

Department of Chemistry, Northwestern University, Evanston, Illinois

Received February 2, 1931

Any survey of chemical literature impresses the reader again with the fact, long recognized, that water is indeed the "universal solvent." As Conant says, "Much important chemistry has been obscured by our slavish devotion to water." Of the work on non-aqueous solvents, the major part has had to do with the physical constants, to the neglect of the nature and type of the reactions and the products formed. It has become increasingly evident, however, that a sound understanding of both the chemistry and the physics of these solutions is essential to a basic and more inclusive knowledge of chemistry.

After the successful application of the ideas of Arrhenius to electrolytic dissociation in aqueous solutions, it appeared reasonable that the results obtainable with other solvents should be capable of similar interpretation. In the early investigations of non-aqueous solutions, however, serious difficulties were encountered. Much of the earlier work is of questionable accuracy and later work has revealed such startling discrepancies, when any attempt was made to reconcile results with the accepted theories, that at the present time few of the desired generalizations can be made.

The simple relations between conductance and concentration found for aqueous solutions do not in general appear when other solvents are used. For certain solutions with dielectric con-

¹ These papers were presented at the Symposium on Non-Aqueous Solutions before the Division of Physical and Inorganic Chemistry at the Eastern Meeting of the American Chemical Society held in Cincinnati, Ohio, September 9, 1930.

stants approaching that of water, the conductance varies with concentration in a way analogous to that observed in aqueous solutions. This is particularly true of ammonia and the amines. In other cases the conductance decreases with increasing concentration and becomes constant at high concentrations. When the solvent has a very low dielectric constant, the conductance increases with concentration until the solution is about molar and then begins to decrease. It is generally stated that the value of Λ_{∞} is greater in aliphatic than in aromatic solutions of the same type. In the case of alcoholic solutions the results obtained are more or less similar to the results obtained with water solutions, and diverge with the increasing complexity of the alkyl group. Numerous measurements have been made in formic acid, acetic acid, ammonia and the amines. Liquid halogens, halogen hydrides, glycerol, benzene, chloroform, hydrogen peroxide, sulphur dioxide, aluminum bromide, sulfuric acid, hydrocyanic acid and many other non-aqueous solvents have been examined.

The classical researches are those of Walden, who examined a large number of substances. Many attempts have been made, particularly by Kraus and Walden, to reconcile the data obtained with the classical dilution laws; the success of these attempts is questionable.

The temperature coefficient of conductivity in non-aqueous solutions is again a function that does not conform to classical laws. In some work done in this laboratory it was found that for the alkyl magnesium bromides in ether solution the equivalent conductivity increases with temperature between 20° and -10°, while for phenyl magnesium bromide in the same range it passes through a maximum.

The dielectric constant of many solvents is known at present with considerable accuracy. The Nernst-Thompson rule relating ionizing power to dielectric constant has been broadly confirmed by experiment. However, we have discovered in recent years other influences promoting ionization, and these appear most markedly when the dielectric constant is small. The dielectric constants of hydrogen bromide and hydrogen

iodide, for example, are low, yet these solvents give excellent conducting media with certain organic acids.

Originally the assumption was made that all reactions are ionic. The curious arrest of many reactions in carefully dried solutions lent support to this generalization. Careful experiments, however, have shown that reactions can take place in non-aqueous solutions that show no conductance. The question of the application of the mass action law to non-aqueous solutions has been studied in detail. In some cases where the solvent is water-like, Ostwald's dilution law is followed. Concentrated solutions of ammonium formate in anhydrous formic acid obey the law. In other cases the Rudolphi dilution law is followed. The equation of Kraus and Bray has been applied to non-aqueous solutions with some success. In many solutions in ammonia the mass action law holds good, and the smaller the ionization the higher the concentration to which the law is applicable.

Little accurate work is on hand relative to the solvated compounds formed when various salts are dissolved in different solvents. Transport numbers in solvents other than water have been determined, but little work has been done here. This field would possibly give us much information as to the type of ionization and the compounds formed. Electrolysis can be carried out in non-aqueous solvents and the results have both scientific and practical value, yet this is a phase of chemistry that until recently has been almost entirely neglected.

Such is the present situation so far as the physical aspects of non-aqueous solutions are concerned. If we are to free ourselves from our thralldom to water and enlarge our conception of chemistry, some detailed study of the chemical reactions taking place in non-aqueous media is required. In such a study the nature of the solvent cannot be neglected, as was formerly done in the study of aqueous solutions.

In recent years many new and brilliant workers have entered this field, stimulated, no doubt, by the newer theoretical considerations and the successful application of interionic attraction theories to strong electrolytes in aqueous solution. It was entirely impossible to reconcile strong electrolytes with van't

Hoff's law of dilute solutions or to the classical mass action law; therefore the original theory has had to be revised. Some of the difficulties have been overcome by considering strong electrolytes as completely ionized and assuming that the electrical forces between the ions account quantitatively for the observed deviations. By far the most successful, although certainly not the first of these interionic theories, we owe to Debye and Hückel. They showed that it was possible to explain in a purely theoretical manner the change in the activity coefficient of ions with concentration. This change can be represented by a square root law when the solution is dilute enough to be regarded as completely ionized. This theory, applied recently to non-aqueous solutions, is promising. We have learned that the ionizing power does not depend entirely on the dielectric constant, but that other factors are perhaps more important. We find that we can classify solvents in four groups: acid solvents such as hydrofluoric acid; basic solvents like ammonia; amphiprotic solvents such as water; and aprotic solvents such as benzene.

One of the main contributions to the chemistry of non-aqueous solvents, certainly from a scientific point of view, has been the enlarging of our conception of acids and bases. Kraus and Franklin have defined acids and bases in an ammonia system. Conant, Hall and Davidson in their work on acetic acid have defined an acid and a base in an acid medium, while Brönsted has perhaps formulated the best definition of these substances for all solvents.

Hall's excellent paper reviews the efforts made to formulate a general definition of an acid and a base. The various methods of obtaining quantitative data on acidity in various solvents are discussed, and the discrepancies in acid strength, as obtained by the different methods, are pointed out. Definite conclusions have been drawn regarding the rôles played by the acidic and basic groups in general. Reactions catalyzed by hydrogen ion are catalyzed by all acid molecules. Likewise all basic molecules have the same properties. The possibility of reducing acidity data, when various solvents are considered, to a quantitative basis seems remote. The establishment of a definite acidity

scale is doubtless the goal of this work but if, as Taylor has pointed out, electric potential difference in different solvents is without meaning, we are still a long way from this "absolute activity."

Davidson points out that our knowledge of the chemistry of acetic acid is quite recent. Although acetic acid is an excellent solvent and is easy to investigate, for years its chemistry was a mystery. He finds that metathetical reactions in this solvent parallel those in water solutions to a great extent in spite of a very different dielectric constant. The main difference is in the solubility relations. Many substances quite soluble in water are insoluble in acetic acid. Acids such as perchloric, sulfuric and hydrochloric are shown to be strongly acid in acetic acid, while the metal acetates are bases and can be used to titrate the acids. The acetate ion in this case shows an avidity for the proton similar to that exhibited by hydroxyl ion. The hydrogen ion activity at the neutral point is, of course, vastly different from a similar neutral point in water solution. The solubility of bases in different solvents varies greatly, just as does that of the metallic bases in water. For example, there is extreme variation in the solubility of the metallic acetates in acetic acid, the acid sulfates in sulfuric acid and the fluorides in hydrofluoric acid. An exception is the case of the metallic sulfides which (excepting NH_4HS) are insoluble in liquid hydrogen sulfide.

Ammonia in acetic acid solution exhibits strong basic properties, whereas ammonia in water does not. This is no doubt due to the instability of $\text{NH}_3\text{H}_2\text{O}$ and the weak basic nature of water, incapable of uniting with proton. Davidson's work points to an explanation of the weak basic nature of water in terms of the instability of oxonium acetate. The formation of sodium zincate when zinc hydroxide is treated with excess of base has a perfect analog in the compound formed when excess sodium acetate is added to precipitated zinc acetate in acetic acid. Copper acetate likewise seems to be amphoteric.

Another neglected acid solvent is hydrogen fluoride, similar to water in its dielectric constant but vastly different in most

other particulars. The alkaline hydroxides and the alkaline earth hydroxides in this solvent increase in solubility with molecular weight, which is analogous to the behavior of similar compounds in water; but in other respects the solvent does not parallel water. Simons has used the acceptor theory of Sedgwick, coupled with the strong acid nature of the solvent, to explain the abnormalities encountered in hydrofluoric acid solutions of salts, acids and alcohols. In the light of the theory here reviewed solvents can be classified according to their ability to accept protons. A strong base accordingly represents one extreme, with hydrofluoric acid at the other extreme, and water intermediate between them. Water would give up protons to ammonia but accept them from hydrofluoric acid, forming an oxonium ion. Hydrochloric acid would give up protons to both ammonia and water but not to hydrofluoric acid, while acetic acid, which is a weaker acid in water, would give up protons to ammonia and water but receive them from hydrofluoric acid. In this solution it would be basic; thus it is evident that the properties of a polar solvent depend not only upon its dielectric constant but also upon its donor and acceptor powers.

Hydrogen sulfide as a potential solvent is placed between water and acetic acid. Its conductance is about 20,000 times less than that of pure water. Most substances give poorly conducting solutions in this solvent. Some substances that are hydrolyzed in water and soluble in liquid hydrogen sulfide are not thiohydrolyzed. No explanation is advanced for this fact, although many esters hydrolyzed in water form analogous sulfur compounds in liquid hydrogen sulfide.

Ammonia solutions have been well studied by numerous workers. Kraus discusses liquid ammonia as a medium for reduction reactions. Ammonia and the amines are unique in their power to dissolve alkali metals. The metals behave as salts, giving, as the negative ion, the electron which is very active chemically. We have in these metallic solutions the strongest type of reducing media. Since water solutions cannot contain a stronger reducing agent than hydrogen, the realm of strong reduction has remained heretofore unexplored. Its investigation

shows many spectacular results. When a salt is added to these metallic solutions, rich in an ammoniated electron, the reaction direction depends on the insolubility of possible reaction products, as well as on the position of the metals in the electromotive series. Here magnesium and aluminum reduce sodium. Metallic alloys of an alkali and an amphoteric metal change to poly-salts that are very soluble and excellent conductors. Positive radicals become free radicals and are then reduced. Metallic and electrolytic conductance exist in the same solution. From the many new types of compounds that appear, it seems that we have veritably discovered a new chemistry.

The application of the Debye-Hückel interionic electrical force theory to non-aqueous solutions is still in the early empirical stage. Probably the best way to attack this problem would be by testing the activities of various types of salts in water-like solvents such as the alcohols. Williams has made such tests and finds that the difference between water and alcohol as solvents is one of degree only. The data, however, reveal the inadequacy of the two premises of Debye and Hückel when applied to non-aqueous solvents. Williams indicates the nature of additional assumptions necessary to reconcile experiment and theory.

Williams also discusses the application to conductance of the Debye-Hückel theory, as modified by Onsager, showing that it can be applied to non-aqueous solutions just as the activity theory was applied, but with less exact results. Deviations from this theory are found in even the simplest types of electrolytes in dilute solutions of methyl alcohol. The results demonstrate the same inadequacies in the assumptions underlying the conductance theory as were found in the activity theory and are to be accounted for chiefly by association and solvation. In effect they limit the applicability of the square root law to the expression of interionic forces. This association also renders it impossible to account for changes in conductance with concentration through changes in ionic mobilities.

Scatchard reviews the different theories of non-electrolytic solutions. He then proposes a modification of Hildebrand's

treatment for use quantitatively. The properties of liquid mixtures can be calculated from certain general assumptions and it is shown that the theory is capable of rather broad application.

Audrieth's paper criticizes the lack of work done on electrolytic deposition in non-aqueous solutions. He reviews the work that has been done in this field with various solvents and points to the need of a generalization for predicting the solvent to be employed in the electrolysis of any given element.

AN INTRODUCTION TO THE CHEMISTRY OF ACETIC ACID SOLUTIONS

ARTHUR W. DAVIDSON

Department of Chemistry, University of Kansas, Lawrence

Received January 12, 1931

I. ACETIC ACID AS A SOLVENT FOR ELECTROLYTES

Acetic acid has been familiar to chemists for so long a time, is so readily obtainable in a pure state, and is a liquid over so wide and convenient a range of temperature (16.6° to 118°), that it seems strange that its properties as a solvent for inorganic substances should have remained, until recently, so little known. But when Raoult (1), in 1884, made a study of freezing point depressions in this solvent, he limited himself almost entirely to organic solutes, and Beckmann (2), reporting its ebullioscopic behavior twenty-three years later, made the statement that acetic acid was not a very suitable solvent for inorganic substances. Whether or not it was these precedents that acted as a deterrent to further investigation, it is, of course, impossible to determine; it is a fact, however, that data concerning the solubilities and reactions of electrolytes in acetic acid remained, for many years, meager and unorganized. Several addition compounds were reported, a few solubilities were determined, and conductivity measurements were made on solutions of the alkali acetates and of several other salts; much of this data was compiled by Walden, in 1924, in his "Elektrochemie Nichtwässriger Lösungen" (3).

Within the last few years, interest in this solvent appears to have revived. In 1926, Webb (4), using the freezing point method, studied the activity coefficients of several salts in acetic acid solution, and in the following year Hall and Conant (5), using an electrometric method for the determination of hydrogen ion activity, showed how the properties of acids and bases in

glacial acetic acid might be studied in a very thorough and interesting manner. Selected references to work in this field up to 1928 were given in the first paper of a series by the present writer (6); in this paper, also, there were reported the results of preliminary experiments, of a qualitative nature, on the solubilities of salts in anhydrous acetic acid. It immediately became evident, not only that this liquid was capable of dissolving a much larger number of salts than had been previously supposed, but also that the chemical properties of these salt solutions were of sufficient variety and interest to repay further study. Without undertaking to give a complete list of soluble salts, it may be stated that these include not only many of the acetates (which are to be regarded as bases in acetic acid, as will be shown later), but a large number of nitrates, halides (particularly iodides), cyanides and thiocyanates as well; in many cases, stable solvates have been isolated in the solid state from these solutions. When to this list are added water, ammonia, and some of the common acids, such as perchloric, sulfuric, hydrochloric, orthophosphoric, and hydrogen sulfide, all of which are readily soluble in acetic acid, it is clear that we have at our disposal a group of acids, bases and salts large and varied enough to enable us to acquire at least a general idea of the chemistry of acetic acid solutions.

II. METATHETIC REACTIONS OF SALTS, ACIDS AND BASES IN ACETIC ACID AS SOLVENT

The early conductivity measurements on salts in acetic acid solution, together with the freezing point determinations of Webb and the electrode potential measurements of Hall and Conant, leave no room for doubt that, in spite of its rather low dielectric constant (about 6 at 25°), typical acids, bases and salts are dissociated into ions in this medium. That the dissociation can hardly be regarded as complete (or that the effect of interionic attraction must be large) is a point which need not concern us here. We should expect, therefore, that ordinary metathetical reactions would proceed smoothly in acetic acid. Experiment showed (6) that such reactions do take place, in many cases as readily as in water, with the formation of slightly soluble or un-

dissociated substances. It appears that practically all salts that are difficultly soluble in water are insoluble in acetic acid also.¹ In accordance with this rule, it was found, for instance, that solutions of soluble salts of the heavy metals gave immediate precipitates upon the introduction of hydrogen sulfide gas; some of the sulfides so precipitated dissolved readily in a dilute solution of hydrogen chloride. (No sulfide precipitates were obtained, however, with salts of the alkali or alkaline earth metals, or of aluminum.) Similarly, a solution of silver nitrate readily yielded precipitates with solutions of soluble chlorides, bromides, iodides, cyanides or thiocyanates, and zinc chloride with a solution of sodium oxalate or of orthophosphoric acid, just as in aqueous solution. It would serve no purpose to extend this list further.

On the other hand, a number of salts which are easily soluble in water are practically insoluble in acetic acid. The most familiar group of salts in this class are the sulfates of the metals, all of which, even those of the alkalies, are difficulty soluble in acetic acid. Using a solution of anhydrous sulfuric acid in acetic acid as the precipitant, a sulfate or an acid sulfate can be precipitated from a solution of almost any soluble metal salt. The acetates of many of the less positive metals are insoluble, and can be precipitated from solutions of soluble salts by the addition of sodium acetate. And as a final example of a reaction which has no close parallel in aqueous solution it may be mentioned that barium nitrate is sufficiently insoluble to be precipitated upon mixing solutions of sodium nitrate and barium iodide.

Certain salts which are known to be only slightly dissociated in aqueous solution give evidence of but slight dissociation in acetic acid also. Thus the very deeply colored ferric thiocyanate is just as readily formed by metathesis in acetic acid as in water. Antimony trichloride and stannic chloride (if the latter may be called a salt) give no precipitates with sulfuric acid or with silver nitrate, presumably due to their being almost undissociated. It may be mentioned here that on mixing stannic chloride and acetic acid, an unparalleled increase in viscosity is observed, the

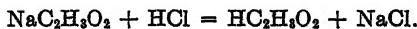
¹ Mercuric iodide, which is fairly soluble in acetic acid, constitutes the only exception to this rule which has so far been observed.

mixture being enormously more viscous than either component in the pure state. This phenomenon has been studied quantitatively by Stranathan and Strong (7), and a solid solvate of stannic chloride has been isolated from such a mixture by the writer (8).

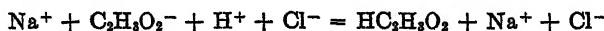
The most interesting group of metathetical reactions, however, consists of those which, while not identical with reactions in aqueous solution, are found to be entirely analogous to them when the nature of the medium is taken into consideration. Outstanding examples of acetic acid analogs of phenomena familiar in aqueous solution are to be found in reactions involving acids and bases. The general concepts of acidity and basicity have been the subject of much discussion within the last few years (9) and need not be considered at length here. In considering acetic acid solutions, we may safely follow Hall and Conant (5), who employ hydrogen ion activity as the criterion of acidity. These authors showed that, on this basis, the familiar acids such as perchloric, sulfuric and hydrochloric have strongly acid properties in acetic acid also. On the other hand, the purely formal analogy between the metal acetates, $MC_2H_3O_2$, in acetic acid, $HC_2H_3O_2$, and the hydroxides, MOH , in water, HOH , is immediately obvious, and might be interpreted as an indication that the metal acetates are bases in the acetic acid system of compounds, just as the metal hydroxides are bases in the water system, or the metal amides in ammonia (10). A formal resemblance of this nature, as Walden (11) has pointed out, is hardly sufficient justification for classifying the substances in question as bases; some real evidence of their basic function is required also. Such evidence was again supplied by Hall and Conant, who showed that sodium acetate (as well as a large number of organic compounds of a basic nature) does indeed decrease hydrogen ion activity when added to acetic acid solutions, and is therefore actually a base in this solvent. Hall and Werner (12) carried out the electrometric titration of sodium acetate with acids in acetic acid solution, and Hall (13) has found that the acetates of the other alkali metals behave in a similar manner. Expressing these facts in terms of Brönsted's conception of acids and bases (14), we might say that in all these cases the actual base is the acetate ion, which shows an avidity

for the hydrogen ion, or proton, similar to that exhibited by the hydroxyl ion.

The process of neutralization, then, takes place readily in acetic acid solutions, and follows the course illustrated by the equation



Since pure acetic acid is probably only very slightly dissociated into ions, its specific conductivity being even smaller than that of water, we may write this in the ionic form (neglecting, as we commonly do in the case of reactions in aqueous solution, the solvation of the ions), as follows:



The occurrence of reactions of this type can be observed in various ways besides the electrometric method which has already been mentioned. It is perfectly practicable to follow the course of the neutralization by means of indicators (15, 16, 17). It must be noted, however, that the hydrogen ion activity at the "neutral" point in acetic acid is far higher than in water, corresponding, according to Hall (13), to a pH value in the neighborhood of zero; so that, for the titration of a strong acid with a strong base in acetic acid, an indicator showing a color change in this region (such as crystal violet, methylene blue or picric acid, to mention only a few) would have to be employed, those commonly used for similar titrations in aqueous solution being entirely unsuitable.

Still another means of observing these neutralization reactions is, of course, available in the event of a marked difference in solubility between the salt produced and one or both of the reactants. Thus the neutralization of sodium acetate by hydrochloric acid is accompanied by the precipitation of sodium chloride. On the other hand, the very insoluble zinc acetate readily dissolves upon the addition of a solution of hydrogen chloride, just as zinc hydroxide dissolves in aqueous hydrochloric acid; while hydroferrocyanic acid, difficultly soluble in acetic acid, dissolves fairly readily in sodium acetate solution.

Further examples of acetic acid analogs of reactions familiar in aqueous solution will be given later.

III. SOLUBILITIES OF ACETATES IN ACETIC ACID

As was pointed out in the preceding section, the metal acetates are the analogs in acetic acid of the metal hydroxides in water. Now, one of the most conspicuous features of the group of hydroxides, in relation to the solvent water, is the enormous variation in solubility from the extremely soluble alkalies, at one end of the scale, to the very insoluble aluminum and ferric hydroxides, at the other. Kendall and coworkers (18, 19, 20) have shown that such variation, far from being peculiar to aqueous solutions,

TABLE I
Solubility of acetates in acetic acid

METAL	SOLVATES ISOLATED	SOLUBILITY, MOLE PER CENT AT 25°	MOLES OF ACETIC ACID PER MOLE OF ACETATE IN SOLID PHASE
K	$\text{KC}_2\text{H}_5\text{O}_2 \cdot 2\text{HC}_2\text{H}_5\text{O}_2$, $\text{KC}_2\text{H}_5\text{O}_2 \cdot \text{HC}_2\text{H}_5\text{O}_2$	12.29	2
Na	$\text{NaC}_2\text{H}_5\text{O}_2 \cdot 2\text{HC}_2\text{H}_5\text{O}_2$, $\text{NaC}_2\text{H}_5\text{O}_2 \cdot \text{HC}_2\text{H}_5\text{O}_2$	7.06	2
Li	$\text{LiC}_2\text{H}_5\text{O}_2 \cdot \text{HC}_2\text{H}_5\text{O}_2$	9.37	1
Ba	$\text{Ba}(\text{C}_2\text{H}_5\text{O}_2)_2 \cdot 3\text{HC}_2\text{H}_5\text{O}_2$, $\text{Ba}(\text{C}_2\text{H}_5\text{O}_2)_2 \cdot 2\text{HC}_2\text{H}_5\text{O}_2$	3.48	3
Ca	$\text{Ca}(\text{C}_2\text{H}_5\text{O}_2)_2 \cdot \text{HC}_2\text{H}_5\text{O}_2$	0.068	1
Zn	None	0.0018	0
Pb	None	31.9	0
Fe(-ic)	None	Practically 0	0
Cu	$\text{Cu}(\text{C}_2\text{H}_5\text{O}_2)_2 \cdot \text{HC}_2\text{H}_5\text{O}_2$	0.166	1
Ag	None	0.094(76°)	0

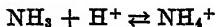
is found in several series of systems of the type $\text{HX}: \text{MX}$. The order of solubility of a series of sulfates in sulfuric acid, or of formates in formic acid, is very similar to that of the corresponding hydroxides in water. More recently, Fredenhagen and Cadenbach (21) have shown the same thing to be true for solutions of fluorides in anhydrous hydrogen fluoride. An explanation of this regularity, in terms of the effect of addition compound formation on solubility, is offered by Kendall in one of the articles just referred to (20). The results of accurate measurements on a number of acetates in acetic acid (17, 19) fall in line with the analogous cases in other solvents. As is shown in table I, where

some of these results are summarized briefly, the acetates of the alkali metals and of barium exhibit marked solvation and are readily soluble, while the solubility of the acetates of most of the less electropositive metals is small, lead acetate constituting a very marked exception.

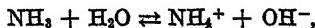
IV. AMMONIA AND WATER AS BASES IN ACETIC ACID SOLUTION

The large group of organic bases will not be considered in this article, but any discussion of basicity in acetic acid solution would be incomplete without mention of the two additional inorganic basic substances, ammonia and water.

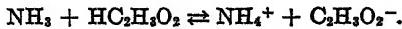
The basic nature of ammonia may be regarded from two different points of view. According to the ideas of Brönsted (14), ammonia is to be regarded as a base because of its tendency to combine with a hydrogen ion, or proton, to form ammonium ion:



More specifically, in aqueous solution, we might write



where NH_3 and OH^- are both bases. A corresponding formulation for ammonia in acetic acid solution would be



Despite the advantages of this viewpoint, too close adherence thereto might, it appears to the writer, have the undesirable effect of obscuring the very close similarity between ammonium compounds and those of the alkali metals, especially potassium. In order to emphasize this similarity, it would seem preferable to formulate the above reactions as follows:



and



Ammonium acetate is, of course, a well-known salt, which is quite stable with respect to ammonia and acetic acid, not only at

room temperature, but up to its melting point, 113°; and it is quite unlikely that it is *completely* dissociated into its ions in acetic acid solution. Moreover, the freezing point curve for ammonium acetate in acetic acid is quite similar to that for potassium acetate (17), and Hall (13) has found that the two compounds behave quite similarly on titration with acids. There is every indication, then, that in acetic acid solution ammonia combines completely with the solvent, and that the resulting ammonium acetate is as strong a base as potassium acetate. Considering the aqueous solution of ammonia from the same point of

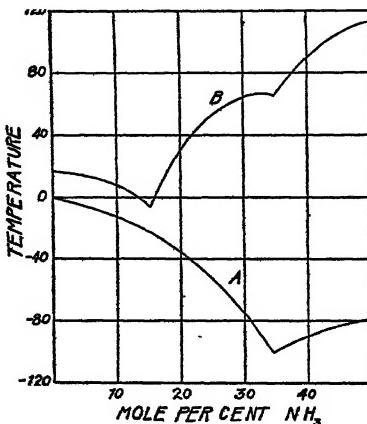


FIG. 1. FREEZING POINT CURVES OF AMMONIA IN WATER (A) AND IN ACETIC ACID (B)

view, the compound ammonium hydroxide should not be regarded merely as a convenient, though probably fictitious, postulate, as some authors have done (22), since it also is capable of existence as a stable solid, though at low temperatures only. In this connection, the freezing point curves of ammonia in water (23) and in acetic acid (17) as solvents may profitably be compared. In figure 1 these curves (for concentrations of ammonia up to 50 mole per cent) are plotted on the same scale, and the enormous difference in stability between the addition compounds $\text{NH}_3 \cdot \text{H}_2\text{O}$ or ammonium hydroxide, on the one hand, and $\text{NH}_3 \cdot \text{HC}_2\text{H}_3\text{O}_2$ or ammonium acetate, on the other, is clearly brought

out. Whatever may be the cause of this difference, it seems altogether reasonable to advance the hypothesis that at very low temperatures (-90° or thereabouts) a solution of ammonium hydroxide might behave in an entirely analogous manner to one of potassium hydroxide. However, the rather flat maximum in the freezing point curve indicates that ammonium hydroxide is somewhat dissociated into ammonia and water even at -79° , and the extent of this dissociation doubtless increases with increasing temperature, so that the weakness of ammonium hydroxide as a base under ordinary conditions may reasonably be ascribed to the instability of the compound with respect to ammonia and water.

Whereas ammonia in acetic acid solution, as has just been mentioned, exhibits strongly basic properties, the closely related substance water, though also a basic solute, is only weakly so. This is indicated both by the potential measurements of Hall and Conant (5) and by observations on the effect of water upon indicators in acetic acid (17). The weakly basic nature of water is accounted for, in terms of Brönsted's ideas, by the hypothesis that, although the water molecule will combine with a proton to form the oxonium ion, H_3O^+ , the tendency for this reaction to take place is considerably less than in the case of the ammonia molecule. Or, if we choose to formulate the behavior of water as a solute in a manner entirely analogous to that used for ammonia, we might write



However, the existence of the addition compound, which may be called oxonium acetate, is open to question, since, unlike ammonium acetate, it is unknown in the solid state at ordinary temperatures. In figure 2 the freezing point curves (up to a concentration of 50 mole per cent of solute) for water (24) and for ammonia (17) as solutes in acetic acid are plotted on the same scale. It is evident that oxonium acetate cannot be isolated as a solid even at very low temperatures. Nevertheless, the identity between the cryoscopic effects of the two substances in moderately dilute solutions, as shown by the exact coincidence of the two

curves in this region, points toward a similar state of solvation at these low concentrations, and makes it seem probable that oxonium acetate is capable of existence *in solution* and that it is actually formed, to some extent, when water is dissolved in acetic acid. Again it seems entirely reasonable to explain the low basicity of water in acetic acid solution as compared to ammonia, in terms of the instability of oxonium acetate as compared to ammonium acetate.

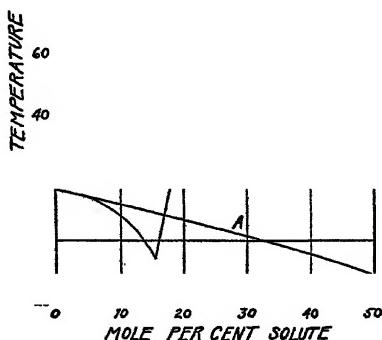


FIG. 2. FREEZING POINT CURVES OF WATER (A) AND OF AMMONIA (B) IN ACETIC ACID

V. THE EFFECT OF SODIUM AND AMMONIUM ACETATES UPON THE SOLUBILITY OF OTHER ACETATES IN ACETIC ACID

When a small amount of sodium acetate solution is added to a solution of zinc chloride in acetic acid,² a precipitate of zinc acetate is formed, the reaction being expressed by the equation



This reaction is, of course, analogous to the precipitation of zinc hydroxide by the addition of sodium hydroxide to an aqueous

² Prepared by heating a solution of hydrogen chloride with an excess of zinc acetate and filtering.

solution of a zinc salt. Now, just as the precipitate of zinc hydroxide dissolves in an excess of sodium hydroxide solution, so does the precipitate of zinc acetate, in acetic acid, dissolve in an excess of sodium acetate solution. Detailed investigation of this phenomenon (25) showed that the analogy between the two cases is far from being merely a superficial one. The solubility curve of zinc acetate in acetic acid solutions containing varying concentrations of sodium acetate, at a constant temperature, is

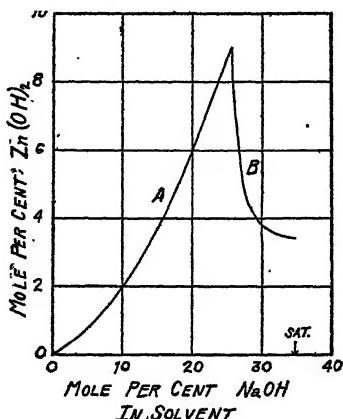


FIG. 3

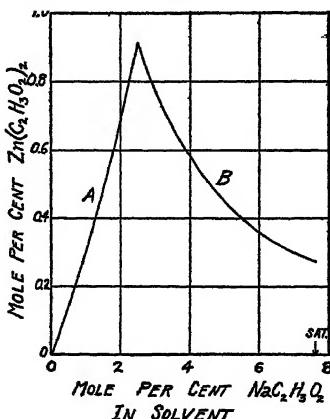


FIG. 4

FIG. 3. EFFECT OF NaOH UPON THE SOLUBILITY OF $Zn(OH)_2$ IN H_2O AT 30°
A—solid phase $Zn(OH)_2$; B—solid phase $Zn(OH)_2 \cdot 2NaOH \cdot 2H_2O$

FIG. 4. EFFECT OF $NaC_2H_3O_2$ UPON THE SOLUBILITY OF $Zn(C_2H_3O_2)_2$ IN $HC_2H_3O_2$ AT 28°
A—solid phase $Zn(C_2H_3O_2)_2$; B—solid phase $Zn(C_2H_3O_2)_2 \cdot 2NaC_2H_3O_2 \cdot 4HC_2H_3O_2$

strikingly similar to the curve for zinc hydroxide in aqueous sodium hydroxide solutions, as may be seen by a comparison of figure 3, plotted from the data of Goudriaan (26), and figure 4, from the investigation (25) just referred to. In each case the solubility of the zinc compound increases with increasing concentration of sodium compound until a maximum is reached,³ at

³ In figure 3 this branch of the curve shows the solubility of zinc hydroxide, although this compound is metastable with respect to zinc oxide under these conditions.

which point a new solid phase appears; beyond this point the concentration of zinc compound in the saturated solution decreases with increasing concentration of sodium compound. The solid phase which appears at high concentrations of sodium compound may be formulated, in each case, as a ternary addition compound of the three components of the solution. The composition of these two ternary compounds is very similar, as is evident from the following comparison: in water— $\text{Zn}(\text{OH})_2 \cdot 2\text{NaOH} \cdot 2\text{H}_2\text{O}$, or $\text{Na}_2\text{ZnO}_2 \cdot 4\text{H}_2\text{O}$; in acetic acid— $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{NaC}_2\text{H}_3\text{O}_2 \cdot 4\text{HC}_2\text{H}_3\text{O}_2$, or $\text{Na}_2\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_4 \cdot 4\text{HC}_2\text{H}_3\text{O}_2$. In the aqueous system zinc hydroxide is said to be amphoteric, and the ternary com-

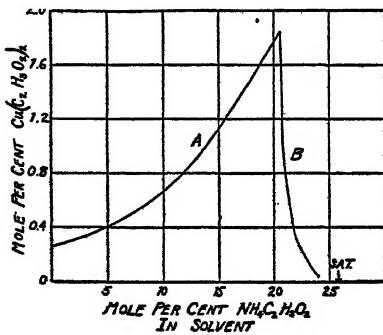


FIG. 5. EFFECT OF $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ UPON THE SOLUBILITY OF $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$ IN $\text{HC}_2\text{H}_3\text{O}_2$ AT 33.5°

A—solid phase $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{HC}_2\text{H}_3\text{O}_2$;
B—solid phase $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{NH}_4\text{C}_2\text{H}_3\text{O}_2 \cdot 4\text{HC}_2\text{H}_3\text{O}_2$.

pound is thought of as a sodium zincate. An answer to the question of whether or not this name, or some modification thereof, such as sodium acetozincate, would also be suitable for the ternary compound in the acetic acid system, must wait upon further investigation of the properties of this substance.

The solubility of cupric acetate in acetic acid, though considerably greater than that of zinc acetate, is still rather low. It was found, however, that cupric acetate dissolves very readily in an ammonium acetate solution, and a quantitative study of this phenomenon (27) again led to interesting results. Figure 5 shows the variation in the solubility of cupric acetate with varying con-

centration of ammonium acetate in acetic acid, at a constant temperature. The solubility of cupric acetate rises to a maximum, at which point a ternary addition compound appears; with further increase in ammonium acetate concentration, the concentration of cupric acetate in the saturated solution falls off. The composition of the ternary addition compound in this case may be represented by the formula $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{NH}_4\text{C}_2\text{H}_3\text{O}_2 \cdot 4\text{HC}_2\text{H}_3\text{O}_2$.

The writer was at first disposed to regard these facts as exactly analogous to the solubility of cupric hydroxide in aqueous ammonium hydroxide solution. There is, indeed, a marked resemblance between the two cases; the solubility of cupric hydroxide increases with increasing concentration of ammonium hydroxide, and, although there is no satisfactory evidence as to the composition of the solid phase in equilibrium with solutions of high ammonia content, the compound $\text{Cu}(\text{OH})_2 \cdot 4\text{NH}_3$ is commonly supposed to be present in the solution. Further consideration, however, has led to a somewhat altered viewpoint. It has been mentioned above that ammonium acetate in acetic acid, unlike aqueous ammonium hydroxide, is as strong a base as the corresponding potassium compound; moreover, qualitative experiments have shown (27) that cupric acetate readily dissolves in potassium acetate solution also. It therefore seems preferable to regard the effect of ammonium acetate on the solubility of cupric acetate in the same light as the effect of sodium acetate on zinc acetate, discussed above, and to seek the water analog of this phenomenon in the effect of the alkali hydroxides, rather than of ammonium hydroxide, upon cupric hydroxide. Although cupric hydroxide is not ordinarily considered to be amphoteric, it is appreciably soluble in concentrated aqueous solutions of alkalies. In fact, Müller (28), in a quantitative study of its solubility in sodium hydroxide solution, obtained a solubility isotherm of the same type as those of figures 3, 4 and 5, with an addition compound which he called sodium cuprite appearing as solid phase at very high concentrations of alkali.⁴ It may be mentioned here that

⁴ The investigation of such systems in aqueous solution is extremely troublesome because of the high viscosity of the concentrated alkali solutions which

many addition compounds of this nature have been obtained from solutions in liquid ammonia, and have been discussed by Franklin (10).

One further property of these cupric acetate-ammonium acetate-acetic acid solutions remains to be noted. On heating them to a temperature of 100° or higher, they exhibit a very marked deepening of their blue color. At the boiling point they attain a very intense shade of violet-blue, which is practically identical with the familiar color characteristic of aqueous solutions containing the so-called cupric ammonia ion. The resemblance is so close that it seems unlikely that it could be merely fortuitous, especially since the cupric acetate-potassium acetate solutions show no color change whatever on heating. It would therefore seem a reasonable hypothesis that at these elevated temperatures a solution of ammonium acetate in acetic acid approaches the state of an aqueous ammonium hydroxide solution at ordinary temperatures; that is, that the ammonia is but loosely held by the solvent, and is therefore available for the formation of other ammonia complexes which are more stable at the temperature in question. If this supposition is correct, then the violet-blue color of the hot cupric acetate-ammonium acetate solutions may be due to a complex ion, $Cu(NH_3)_n^{++}$, very similar to that which gives rise to the same color in aqueous solutions.

Several other facts have been observed which also seem to point toward a loosening of the bond between dissolved ammonia and acetic acid at temperatures in the neighborhood of the boiling point of the solvent. For instance, silver chloride does not dissolve in an acetic acid solution of ammonium acetate in the cold, as it does in aqueous ammonia, but does dissolve when the mixture is heated to boiling. Similarly, mercurous chloride, which is quite insoluble in acetic acid, shows no change of color when ammonium acetate is added at room temperature, but turns gray on being heated. And finally, the fact that the distillate from a concentrated solution of ammonium acetate in acetic acid

have to be employed, and the difficulty of obtaining substances like cupric hydroxide or zinc hydroxide in crystalline forms of constant composition. In the acetic acid systems, these difficulties do not arise.

contains perceptible quantities of ammonia (29), shows that the vapor pressure of ammonia in such a solution, which must be practically zero at room temperature, becomes appreciable at the boiling point of the solution. The conclusion may be drawn, then, that while ammonium acetate in acetic acid at 25° is comparable to potassium hydroxide rather than to ammonium hydroxide in aqueous solution, yet above 100° it begins to exhibit properties similar to those of an aqueous ammonium hydroxide solution at low temperatures.

Some of the phenomena which have been discussed in this article have so far been studied only superficially, and further investigation will be necessary for a better understanding of the facts observed and a more thorough confirmation of the hypotheses advanced; while many other possible reactions and relationships have not been touched upon at all. Nevertheless, it appears to the writer that the value of the study of non-aqueous solutions, as a means of broadening our perspective of general chemistry, is further demonstrated by these first steps toward the development of the chemistry of acetic acid solutions.

REFERENCES

- (1) RAOULT: Ann. chim. phys. [6] 2, 66 (1884).
- (2) BECKMANN: Z. physik. Chem. 57, 129 (1907).
- (3) WALDEN: Elektrochemie Nichtwässriger Lösungen, J. A. Barth, Leipzig (1924).
- (4) WEBB: J. Am. Chem. Soc. 48, 2263 (1926).
- (5) HALL AND CONANT: J. Am. Chem. Soc. 49, 3047 (1927).
- (6) DAVIDSON: J. Am. Chem. Soc. 50, 1890 (1928).
- (7) STRANATHAN AND STRONG: J. Phys. Chem. 31, 1420 (1927).
- (8) DAVIDSON: J. Phys. Chem. 34, 1215 (1930).
- (9) See HALL: J. Chem. Education 7, 782 (1930) for a recent summary of modern viewpoints. The theory of acidity is discussed also by HAMMETT: J. Am. Chem. Soc. 50, 2666 (1928).
- (10) FRANKLIN: J. Am. Chem. Soc. 46, 2137 (1924).
- (11) WALDEN: Salts, Acids and Bases, pp. 156-159. McGraw-Hill Book Co., Inc., New York (1929).
- (12) HALL AND WERNER: J. Am. Chem. Soc. 50, 2367 (1928).
- (13) HALL: Private communication, for which the author desires to express his appreciation.
- (14) BRÖNSTED: Chem. Reviews 5, 231 (1928), and previous papers.
- (15) CONANT AND HALL: J. Am. Chem. Soc. 49, 3062 (1927).
- (16) HAMMETT: J. Am. Chem. Soc. 50, 2666 (1928).

- (17) DAVIDSON AND McALLISTER: J. Am. Chem. Soc. **52**, 507 (1930).
- (18) KENDALL AND DAVIDSON: J. Am. Chem. Soc. **43**, 979 (1921).
- (19) KENDALL AND ADLER: J. Am. Chem. Soc. **43**, 1470 (1921).
- (20) KENDALL, DAVIDSON AND ADLER: J. Am. Chem. Soc. **43**, 1481 (1921).
- (21) FREDENHAGEN AND CADENBACH: Z. physik. Chem. **146A**, 245 (1930).
- (22) HAMMETT: Solutions of Electrolytes, p. 96. McGraw-Hill Book Co., Inc.
New York (1929).
- (23) ELLIOTT: J. Phys. Chem. **28**, 887 (1924).
POSTMA: Rec. trav. chim. **39**, 515 (1920).
- (24) FAUCON: Ann. chim. phys. [8] **19**, 70 (1910).
- (25) DAVIDSON AND McALLISTER: J. Am. Chem. Soc., **52**, 519 (1930).
- (26) GOUDRIAAN: Rec. trav. chim. **39**, 505 (1920).
- (27) DAVIDSON AND GAISSWOLD: Unpublished work.
- (28) MÜLLER: Z. physik. Chem. **105**, 73 (1924).
- (29) DAVIDSON: Trans. Kansas Acad. Sci. **31**, 60 (1928).

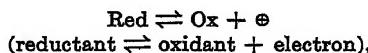
ACID-BASE EQUILIBRIA IN NON-AQUEOUS SOLVENTS WITH PARTICULAR REFERENCE TO GLACIAL ACETIC ACID

NORRIS F. HALL

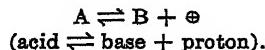
Department of Chemistry, University of Wisconsin, Madison

Received January 31, 1931

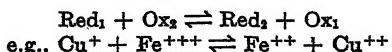
Acids and acidity have taken on a new theoretical importance in the last thirty years, because, after the rôle of the hydrogen ion as the common carrier of acid properties had become established, it was discovered that this hydrogen ion or proton, besides being the lightest atomic particle was also *simple* in a way entirely unique among atoms, and *fundamental* in a way shared only with the electron. It is thus possible to say that just as oxidation-reduction reactions are uniquely characterized by electron exchange between molecules, and may be defined by the general equation



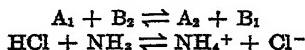
so acid-base or *protolytic* reactions may be defined by the general equation (Goldschmidt, Brönsted)



In actual practice, however, chemical reactions are always more complex than this and the typical "redox" equilibrium involves at least two oxidants and two reductants.



Similarly, acid-base reactions in general involve such an exchange as



The example chosen involves us at once in the question of the advantages and disadvantages of the proposed definition. It is seen that to be consistent we must give the name "acid" to all molecules of the A type which release a proton under the conditions of our study, regardless of their charge, and the name "base," or "protophile," to all which accept protons. This results in calling ammonium ion an acid, chloride ion a base (though a very weak one), and in saying that potassium hydroxide is not itself a base although it contains the strong base hydroxyl ion.

Some hostility is naturally aroused in many minds by such a proposal, and it seems fair to say that chemical opinion is divided at the present time on its desirability. On the one hand are those who prefer to retain the customary definition of a base as something that in water gives hydroxyl ions, in spite of the admittedly partial, inadequate, and one-sided character of this formulation. On the other side we have such suggestions as Germann's (15), or Lewis's (41), which would extend the concept of acids to include such things as phosgeno-aluminum chloride COAl_2Cl_8 , or make "acids" and "bases" synonymous with Sidgwick's (49) "acceptor" and "donor" molecules, respectively.

Brönsted (8), it seems to me, has satisfactorily defended his proposal in the following terms: "If we seek, with these considerations as a background, a more general and more precise definition of acid and base, we recognize that such a definition must in the first place attribute the characteristic acid-base properties to the molecules of acids and bases themselves, (i.e., not to their solutions). Second, it must be a definition which relates the ideas acid and base to each other in a more logical way than has hitherto been the case. It must further give an illuminating explanation of the peculiar character of these substances, and finally we may say that since acids and bases are found so universally in chemical systems, the definition of these substances must be formulated independently of the solvent."

In addition to elegance and logical consistency, one may urge in favor of Brönsted's definition that it has led to new work of importance. This is particularly true in the field of reaction velocity studies, where the recognition of the hydronium and

hydroxyl ions, H_3O^+ and OH^- , not as uniquely important substances, but as members of the classes acid and base, has led to the correct evaluation of the rôle of other acids and bases in protolytically catalyzed reactions (6). Thus Brönsted and his students have shown that the velocity of a reaction catalyzed by H^+ ion is a function of the concentrations and acidity constants of all the acid molecules present, and not simply of the hydrogen ion activity of the solution. The same has also been shown to be true of reactions catalyzed by bases.

Recent papers by Kilpatrick (38, 39), Pedersen (45), and Grove (24), may be mentioned among others as examples of the utilization of these ideas.

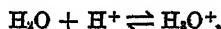
SOLVENTS

When we consider the rôle of the solvent in acid-base equilibria we recognize that solvents may be roughly classed from this standpoint as predominantly acidic, predominantly basic, amphiprotic, or indifferent. (By taking account of the dielectric constant Brönsted (7) increases these four classes to eight.)

Thus we recognize water as amphiprotic since it is capable of the acid reaction



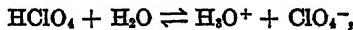
and the basic reaction



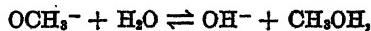
or, what amounts to the same thing, it is self-dissociated to an appreciable extent according to the scheme



In this solvent, therefore, all very strong acids are completely decomposed



as are all very strong bases



while the protogenic and protophilic character of very weak acids and bases is largely masked by the overwhelming prominence of the similar properties of water. A solvent of opposite or *aprotic* character is represented by benzene, which neither gives up nor takes up protons to any considerable extent. Acid solvents which have been more or less thoroughly studied are hydrocyanic acid, phenol, and acetic, formic, hydrofluoric and sulfuric acids. Predominantly *basic* solvents are ammonia and the amines.

Hammett (29) has given a highly interesting discussion of the rôle of the properties of the solvent in determining acidity, and has clearly distinguished between the inferences that may legitimately be made from colorimetric work with indicators of various types and from electrometric measurements. Numerous papers of Hantzsch (31, 31a, 31b, 33) should also be cited in this connection, as well as the recent work of Schwarzenbach (48), Fredenhagen (12, 13, 14) and Rabinowitsch (46). Bjerrum's (3, 4) studies of ion-partition coefficients present a novel method of attack on the problem of relative acidities in different solvents.

Aprotic solvents

Let us first consider the phenomena in relatively indifferent or aprotic solvents. Examples of these are the paraffin hydrocarbons, benzene and chloroform. In these solvents the levelling, or degrading, action which water exerts on strong acids and bases is absent, and we may expect the true or intrinsic relative strength of the stronger acids to come to the fore much more clearly than in water.

In benzene, acids and bases may probably be regarded as present as such—though perhaps associated—and not to any great extent as ionized solvates. Brönsted has shown how such solutions may be studied. If two substances are added to benzene such that an equilibrium of the following type is established



then

$$\frac{C_{A_2} \times C_{B_1}}{C_{A_1} \times C_{B_2}} = \frac{K_{A_2}}{K_{A_1}} = \frac{K_{A_1}}{K_{A_2}}$$

where K_{A_1} and K_{A_2} are the acidity constants of the two acids concerned and K_{A_1} , K_{A_2} are the corresponding concentration dissociation constants. This follows from the definition of the respective constants:

$$K_{A_1} = a_{H^+} \frac{C_{B_1}}{C_{A_1}} \text{ and } K_{A_1} = C_{H^+(sol)} \frac{C_{B_1}}{C_{A_1}}, \text{ etc.}$$

If now one of the substances has at least one colored form—i.e., is an indicator acid or base—the ratio of the concentrations of two of its forms may be determined from the color of the solution, and the strength of a colorless acid may be compared with that of the indicator acid by noting the concentration ratio of, for example, acetic acid to an acetate, which is necessary to produce a given color in the solution.

In this way Brönsted has made a very instructive comparison of the strengths of various uncharged and cation acids with those of a series of indicators in benzene, and arranged these results in a table of relative acid strengths. An inspection of Brönsted's table brings out the following points: for acids of the same charge type the order of strength is nearly the same in benzene as in water; the positively charged acids appear very much stronger in relation to the uncharged, than is the case in water. These results are to be expected from the simple electrostatic theory according to which a separation of unlike charges (ordinary electrolytic dissociation) will occur less readily in a solvent of low dielectric constant, while an equilibrium of the type



will be relatively unaffected by dielectric constant change. (See also Hammett (29)). In order to bring out more clearly the significance of Brönsted's table, I have amplified his list of acids by writing after their names the values of their negative logarithm-

mic dissociation constants; these are arranged in two columns according to the charge type of the acid concerned.

It is evident that the constants fall into two series, that of the cation acids being displaced in the direction of increasing strength, relative to the uncharged acids. Certain irregularities in the

TABLE I
Brönsted's acidity series determined colorimetrically in benzene

SUBSTANCE	VALUE OF pK FOR ACID	
	A°	A^+
1. Hydrochloric acid.....	-7.4	
2. Methyl red.....	4.9(?)	
3. Dimethyl yellow.....		3.5
4. Trichloroacetic acid.....	0.7	
5. Dichloroacetic acid.....	1.3	
6. Pieric acid.....	0.3	
7. <i>o</i> -Nitrobenzoic acid.....	2.3	
8. Chloroacetic acid.....	2.9	
9. Salicylic acid.....	3.0	
10. Bromophenol blue.....	4.1	
11. β -Dinitrophenol.....	3.7	
12. <i>o</i> -Chlorobenzoic acid.....	2.9	
13. Neutral red.....		6.9
14. <i>m</i> -Chlorobenzoic acid.....	3.8	
15. Bromoresol green.....	4.7	
16. Benzylammonium ion.....		9.4
17. Formic acid.....	3.7	
18. Phenylacetic acid.....	4.3	
19. Benzoic acid.....	4.2	
20. Acetic acid.....	4.7	
21. Isoamylammonium ion.....		10.6
22. Bromoresol purple.....	6.3	
23. Piperidinium ion.....		11.1
24. Bromothymol blue.....	7.0	

observed order are attributed by Brönsted to the effect of "zwitterion" structure of the "uncharged" forms. The strongest acid investigated by Brönsted was hydrochloric, of which Wynne-Jones (51) estimates the true constant in water to be $10^{7.1}$. (Schreiner (47) gives 10^4 – 10^6). There is a variety of evidence (16, 18, 31, 33, 34, 35, 47) that hydrobromic and hy-

driodic acids are stronger still, and that perchloric acid is the strongest of all the common acids, though possibly none of them is *completely* dissociated in water. These great differences in strength are practically obscured in water by the conversion of all the acids of this class into the weaker acid H_3O^+ , whose acidity constant is conventionally fixed at 1 by the ordinary definition of proton activity in aqueous solution.

To the German organic chemist, A. Hantzsch, we owe much of our knowledge of the relative strength of the strongest acids, and he has insisted for years on the existence of these differences in relative strength just mentioned. Typical of Hantzsch's investigations are those recently published with Voigt (33) on "the determination of the acidity of undissociated acids." The experiments fall into two parts. In the first the authors prepare the dimethyl yellow salts of the acids studied and dissolve them in a given solvent, diluting with the solvent until the indicator turns color. It is supposed that the dilution at which the color change occurs, and at which presumably the reaction $\text{BH}^+ \text{X}^- \rightarrow \text{B} + \text{HX}$ has reached a certain degree of completeness, is an approximate measure of the strength of the acid in the solvent concerned. The relative dilutions necessary in dry chloroform vary from 1 for acetic acid to 95,000 for trichloroacetic acid, while the dissociation constants as ordinarily measured in water show only an eleven thousand fold variation.

Hantzsch attributes this difference to the "levelling down" influence of water on the stronger acids. If the chloroform is moist the strength of the stronger acids appears relatively less, which is in harmony with this view. Hantzsch regards ether as a much less indifferent solvent than chloroform, and one which forms well-defined solvates with the dissolved acids; thus promoting the decomposition of the salt. He finds, in other words, that a much smaller volume of ether than of chloroform is necessary to decompose completely a given quantity of a particular salt. The necessary dilutions vary from 1/17th to 1/80th of the corresponding figures for chloroform. Added water has much less effect on ether than on chloroform.

When the strongest acids are examined it is found that their

salts with dimethyl yellow are so stable as to be decomposed only at impractically large dilutions in dry chloroform, while in both dry and moist ether-chloroform mixtures, and in moist chloroform, the necessary dilutions are measurable and indicate that the strength of the strong acids falls off in the order perchloric, hydrobromic, hydrochloric, nitric, as much similar previous work has shown. The authors then find in the more weakly basic indicator dianisalacetone a suitable substitute for dimethyl yellow in dry chloroform, and find in fact that hydrobromic, hydrochloric, and trichloroacetic acids show diminishing strength in the order named. (Nitric, perchloric and hydriodic acids could not be measured for special chemical reasons.) The study of these three acids was then extended to the solvents benzene, toluene and tetrachloroethane, with the surprising result that in benzene and toluene, hydrochloric acid appeared slightly weaker than trichloroacetic acid. As this result is in direct contradiction to that of Brönsted reported above, one must either suspect some special chemical reaction of this indicator with the acids and solvent concerned, or question the reliability of one or both of the methods as a true measure of relative acid strength. Both Brönsted and Hantzsch clearly recognize the provisional and approximate character of their results, and point out the more obvious uncertainties by which they are affected.

Schwarzenbach (48) has called attention to a phenomenon of "self buffering," which is to be expected in certain concentration ranges when acids are dissolved in aprotic solvents, and also to the fact that in certain of these solutions the molar conductance should decrease with dilution. He believes that both of these effects are shown by his own measurements on ether solutions.

Basic solvents

(a) *Ammonia.* The early work of Franklin, Kraus, Cady and others showed that the ammonium salts function as acids in this solvent, that many acids relatively weak in water appear to exhibit a relatively high degree of dissociation in ammonia, and that indicators such as phenolphthalein are affected by the NH₃.

ion in the same way as by OH^- in water. Quite recently Zintl and Neumayr (52) have determined the E.M.F. of concentration cells with transference, using quinhydrone electrodes, and have obtained values consistent with equations developed by Bjerrum and others.

(b) *Organic amines.* Aniline and *p*-toluidine as solvents for acids and bases have been studied by Heinrich Goldschmidt and his coworkers Reinders, Bandke, Salcher, and Bakscht (1, 20, 22, 23), who found that aminolysis of salts was more extensive in *p*-toluidine than in aniline, as would be expected from the relative strength of these bases in water. They also found that the order of apparent strength of acids and bases was the same in these two solvents as in water but that the strongest acids, as mentioned above, show widely different strengths. The ratio of apparent strengths of the strong acids in the two solvents is nearly constant (the velocity constant of the acid-catalyzed reaction is about nine times as great in aniline as in *p*-toluidine), but it falls to about half its value for the weaker *o*-nitrobenzoic acid. When different bases are compared in the two solvents it is found that the salts of the stronger bases are both absolutely and relatively less solvolyzed in aniline than in *p*-toluidine.

The author's E.M.F. measurements in pyridine (unpublished) have also shown that in this solvent the apparent strengths of the strong acids differ widely. This is presumably due to the enormously large salt effects to be expected in solvents of low dielectric constant, and appears to be in harmony with the views of Hammett in the paper previously referred to.

(c) *Alcohols.* Goldschmidt and Mathiesen (21), completing and extending earlier studies by Goldschmidt and others (17, 19), report the solvolytic constants for a variety of bases in ethyl and methyl alcohols. These constants are usually smaller in the alcohols than in water and always smaller in methyl than in ethyl alcohol, so that they seem to reach a minimum value in methyl alcohol, a rather surprising result. They also found that the addition of water always reduces the alcoholysis of a salt, and to about the same degree for salts of different acids with the

same base. The effect, however, differs in magnitude according to the base employed.

One may conclude from Goldschmidt's results that the dissociation constant of a cation acid is usually smaller, but sometimes larger, in ethyl alcohol than in water. The ratio of the two constants varies from 0.06 to 6.0. This is in harmony with the expectation referred to above, that the constant of a cation acid should be substantially unchanged on changing solvents. Goldschmidt also determined the dissociation constants of numerous acids in alcoholic solution by conductivity methods, and found that in general an uncharged acid has a dissociation constant about 10^6 times smaller in alcohol than in water. Larsson (40) recalculated and in part repeated and confirmed Goldschmidt's results, and also made E.M.F. measurements of cells with liquid junctions which he interpreted as confirming the conductivity figures very satisfactorily.

Michaelis and Mizutani (42, 43, 44) studied the E.M.F. of cells consisting of a saturated aqueous calomel electrode and a hydrogen electrode in an aqueous alcoholic solution of a half-neutralized acid or base. Saturated aqueous potassium chloride formed the liquid junction in their cells. They calculated at definite percentage compositions of the water-alcohol mixtures the apparent acidity constants of numerous uncharged and cation acids. In most cases an extrapolation to pure water and pure alcohol was possible. From their results one may infer, neglecting the junction potential, that the acidity constant of an uncharged acid is usually about 10^3 times smaller in ethyl alcohol than in water, while that of a cation acid is about 10^2 times larger.

Since in any solvent

$$K_A = \frac{C_{H^+(sol)} \times C_B}{C_A} = K_{Ac} \cdot K_{Bas(solvent)}$$

Brönsted (6) has been able to calculate from certain of these results combined with others of Bjerrum (5) that K_{Bas} for methanol is about 350 times smaller than for water, while two independent calculations show that ethanol is 330 and 600 times as weak a base as water.

Similar calculations also show that pure methanol is 2.5 times and ethanol 160 times as weak an acid as water, but all these figures are affected by numerous uncertainties. In particular, the physical meaning of Brönsted's functions K_{A_c} and $K_{B_{as}}$ for any substance in a non-aqueous or even a concentrated aqueous medium depends on the possibility of measuring relative hydrogen ion activities, which Taylor (50) and Guggenheim (25) have recently reminded us are fundamentally inaccessible to experimental attack.

Bishop, Kittredge and Hildebrand (2) used the hydrogen electrode for titrations in ethanol and emphasized the effect of the low self-ionization of the solvent on the titration curves.

(d) *Ether*. This solvent is distinctly less basic than the alcohols, but more so than the hydrocarbons. In addition to the studies of Hantzsch already referred to, we may mention certain measurements and titrations by Schwarzenbach (48), using hydrogen electrodes.

Acid Solvents

(a) *Acetic acid*. If we now turn to glacial acetic acid as a typical acid solvent, we find that acid-base equilibria have been studied in it primarily by means of titration curves.

Conant and Hall (10, 27) found that the stronger inorganic acids and the sulfonic acids, all of which are quite soluble in the medium, could be used as titrants for basic solutions such as those formed by the organic amines or by the acetates of metals. Potentials reproducible within a few millivolts were obtained by the use of a simple cell containing liquid junctions, in which an aqueous calomel electrode served as a reference, and the indicator electrode was a platinum foil in an acetic acid solution of quinhydrone, or better, chloranil and its reduction product. The bridge between the reference electrode and the solution being titrated was a stoppered glass siphon containing a concentrated solution in acetic acid of a salt such as lithium chloride.

Since the extent of solvolysis of a given salt depends on the basic nature of the solvent, it is to be expected that largely unsolvolyzed salts of weaker bases can be formed in acetic acid than

in water, as indeed is well-known in organic chemical practice. This point of view was summarized by means of a diagram (figure 1) and discussed in the first papers of this series, which should be consulted for details (27). Later Hall and Werner (28) showed the same thing by a comparison of the titration curves of a weak

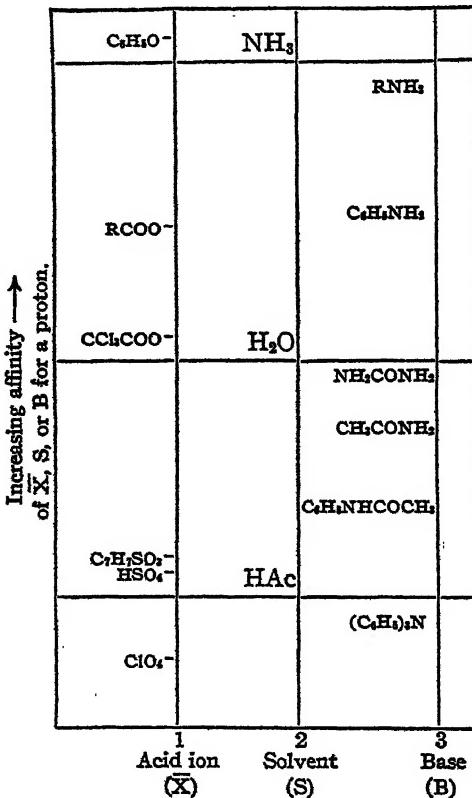


FIG. 1. DIAGRAM ILLUSTRATING THE RELATIONSHIP OF ACIDS AND ANHYDRO BASES IN A VARIETY OF SOLVENTS

base (acetoxime) in three different solvents (figure 2). It was also found that in this solvent, as Hantzsch had shown to be the case in many others, the relative strengths of the strong acids differ widely, so that the same base will in general give a different titration curve with each acid used. Figure 3 shows the po-

tentials of a chloranil electrode in a solution of acetic acid, either alone or with the addition of various bases, when perchloric, sulfuric, and hydrochloric acids respectively are added to the solution.

Most of the later titrations have been made with perchloric acid because of its strength and because it is monoprotic. Figure

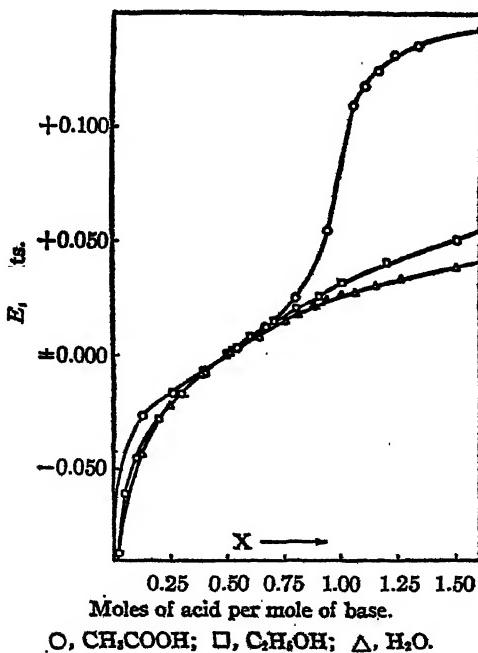


FIG. 2. EFFECT OF THE SOLVENT IN THE TITRATION OF A WEAK BASE (ACETOXIME) WITH PERCHLORIC ACID

4 shows the curves obtained at 0.05 M concentration with some fifty organic amines and oxygen bases. These titration curves with perchloric acid fall into three classes according to base strength, like the titration curves obtained in water, and the "strong base" curves have the same shape as in water solution. The weaker bases, however, give curves that are too flat, presumably because of the change of ionic strength during titration.

Conant and Werner (11) show that, as in many other cases,

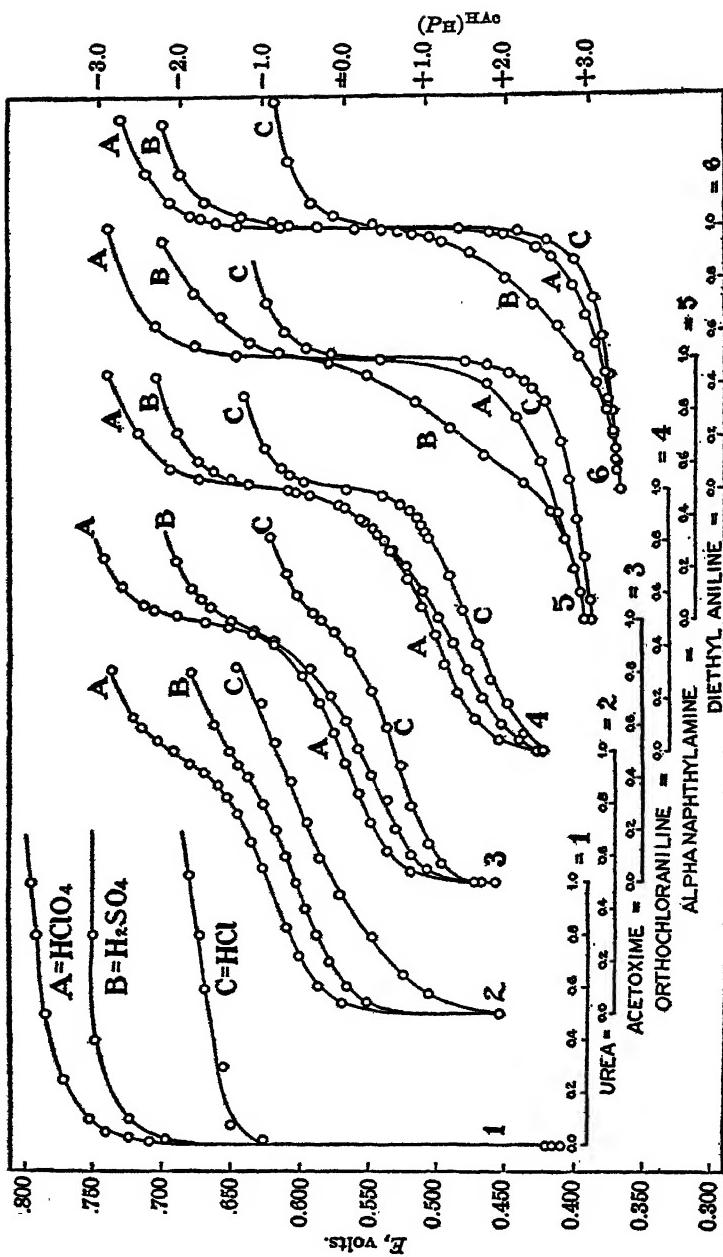


FIG. 3. COMPARISON OF HClO_4 , H_2SO_4 AND HCl AS TITRATING ACIDS
 Abscissas are moles of acid added per mole of base. The curves in group 1 represent addition of acid to the pure solvent. With the stronger bases (5 and 6) the second hydrogen of H_2SO_4 (B) becomes active. The curves in group 1 begin on the basic side of the neutral point due to accidental contamination with traces of basic material.

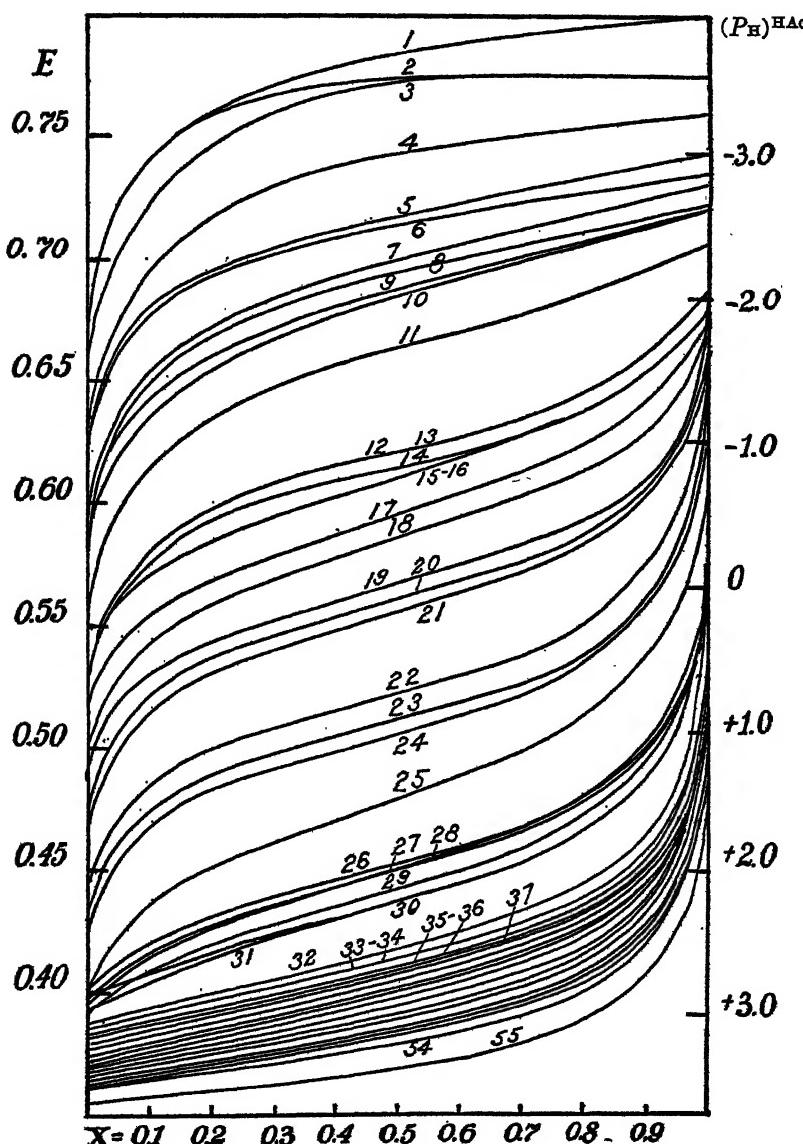


FIG. 4. TITRATION CURVES OF 0.05 M BASES IN GLACIAL ACETIC ACID
Note the three classes: very weak (1-11); weak (11-31); strong (32-55). No. 55 is anomalously low. No. 11 is exceptionally steep.

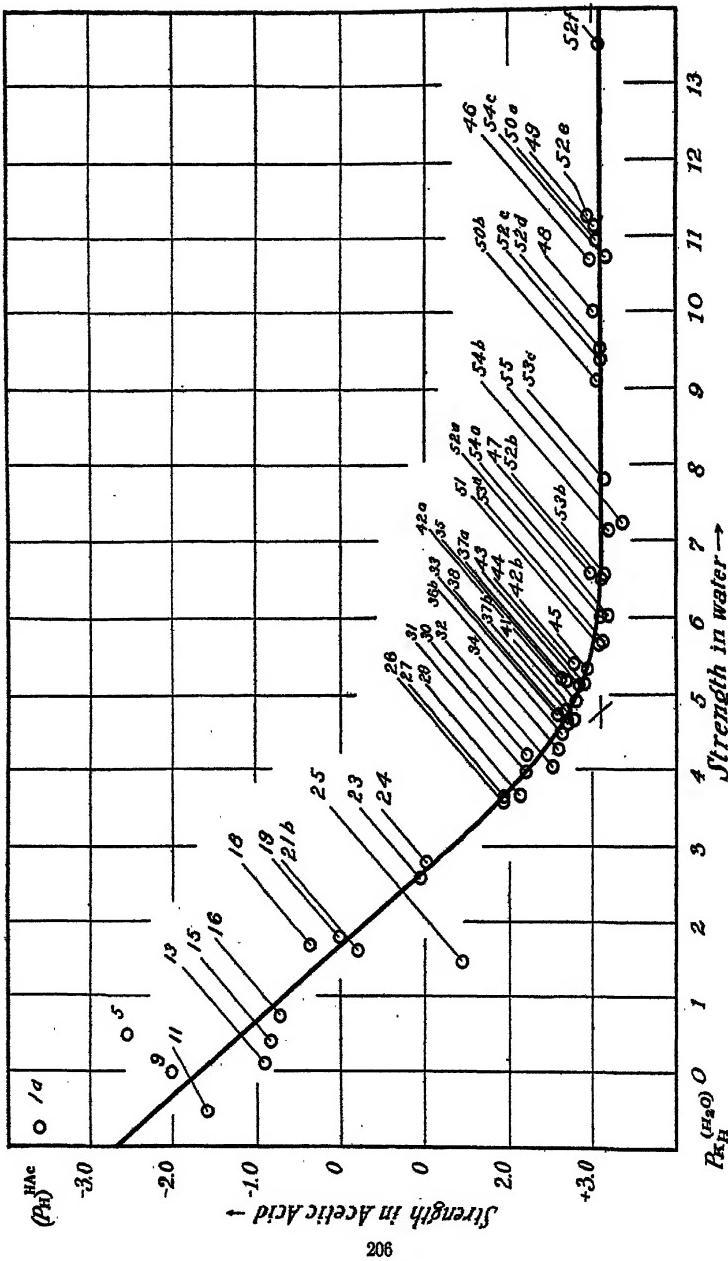
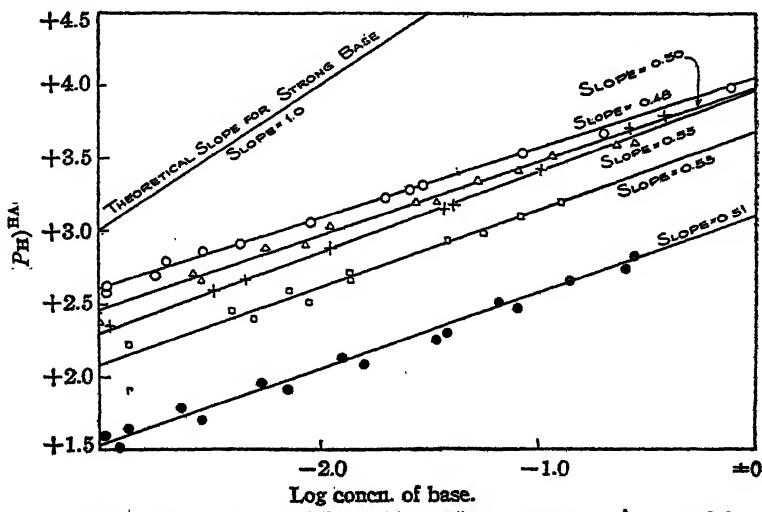


FIG. 5. COMPARATIVE STRENGTH OF BASES IN ACETIC ACID AND IN WATER

these anomalies largely disappear when the ionic strength is maintained nearly constant by a large concentration of added salt.

Similar points on the titration curves of different bases should measure the relative acidity constants of the bases in the solvent, and according to theory these should be nearly proportional to the similar constants in water solution. Because, however, of the strongly acid character of the solvent a much weaker base may be expected to react completely with it than is the case in



O, Diethylaniline; +, pyridine; □, sodium acetate; Δ, guanidine;
●, o-chloro-aniline.

FIG. 6. $(P_K_H)^{HAc}$ -DILUTION CURVES OF BASES IN ACETIC ACID

water, where none of the ordinary amines can be called strong bases.

Both of these points are brought out by figure 5, in which the logarithmic strength constants in the two solvents are plotted against each other for fifty bases. It is seen that bases stronger than aniline ($P_K_H(H_2O) = 4.75$) are completely strong in acetic acid. (For the names of the bases and other details the reader is referred to a recent paper (26) by the author.) In spite of this complete reaction with the solvent, solutions, even of the strongest

bases, do not show conductance or voltage changes on dilution which suggest a high degree of dissociation in the ordinary sense. In fact, the acidities of the solutions change on dilution in just the same way as those of weak bases in water, as though the electrolyte obeyed the dilution law and the activity coefficients of the ions formed remained constant. If the strong base acetates are completely ionized at all concentrations, then the activity coefficient of the acetate ion (not its logarithm) varies as the inverse square root of the salt concentration. These facts are exhibited in figure 6, in which the apparent "pH" of various dilutions of the bases is plotted against the logarithm of the dilution. The points lie on straight lines with a slope of $1/2$, as is to be expected for weak protophiles (28).

In a paper which has just come to hand, Isgarischew and Pletenew (37), working at Moscow, have measured the hydrogen electrode potentials of various concentrations of sodium acetate in dry acetic acid. Allowing for the different electrodes used, their values may be compared with those of Hall and Werner, with the result that neither the actual values of the potentials nor the type of their variation with concentration agrees with the earlier measurements. Further investigation of this discrepancy is very desirable. The Russian investigators are led by their results to the surprising conclusion that pure acetic acid is dissociated "in sehr bedeutendem Masse" into hydrogen and acetate ions, a view difficult to reconcile with a specific conductance of 10^{-7} to 10^{-8} (36).

With Bramann (9), Conant has investigated the catalysis in acetic acid solution of the acetylation of β -naphthol. The logarithm of the velocity constant is shown to decrease linearly with increasing apparent pH of the medium, until a minimum is reached, after which further increase in pH causes a linear increase in the logarithm of the rate constant. This result can be interpreted simply, following the lines of the similar studies of Brönsted in water solution. It appears that the rate depends on the concentration and strength of all the acids and bases present, and that the reaction is catalyzed both by acids and by bases, such as acetate ion.

Acetic acid is thus again shown to be, as Professor Davidson's paper in this symposium has made clear, a solvent worthy of study from many points of view.

(b) *Formic acid.* In a very interesting paper which has just appeared, Hammett and Dietz (30) report titrations of sodium formate and other basic substances in anhydrous formic acid. Quinhydrone electrodes were used, and the titrating agent was

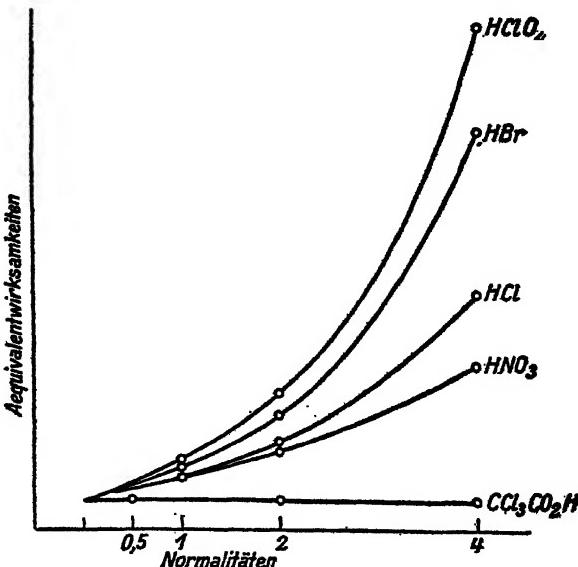


FIG. 7. THE CHANGE WITH CONCENTRATION OF THE EFFECT PER EQUIVALENT OF ADDED ACID ON THE VELOCITY OF INVERSION OF SUCROSE

This figure is taken from an article by Hantzsch and Weissberger: Z. physik. Chem. 125, 251-63 (1927).

benzene sulfonic acid. Curves of the theoretical form for strong electrolytes were obtained, and it was possible to calculate the ion product constant of the solvent for different values of the salt concentration. A high and constant ionic strength was maintained during the titrations. Such weak bases as triphenylcarbinol, benzalacetophenone and urea appeared to be completely dissociated in formic acid, while ether and water showed hardly any basic properties.

If we now return for a moment to the general rôle of the solvent in these reactions, and to the behavior of the strongest acids in various solvents, we may note that even in water itself, if the solution is sufficiently concentrated, the individual behavior of the strong acids comes out very clearly. This has been shown by Hantzsch and Weissberger (34) in studies of the rate of inversion of cane sugar, and is exhibited in figure 7, where the relative increase in the velocity of inversion per equivalent of acid present is plotted against the normality of the solution. It is seen that the relative effect of an equivalent of the acid increases rapidly with the intrinsic strength as inferred from the work in non-aqueous solvents. Studies of the activities of hydrochloric acid, sulfuric acid, etc. in concentrated aqueous solutions should also be mentioned in this connection.

Pure liquid acids themselves represent limiting cases of concentrated acid solutions. Such liquids show specific conductances ranging from 10^{-2} (for sulfuric acid) to 10^{-13} for isovaleric and caprylic acids (36). This conductance is presumably to be attributed to a self-ionization of the type $2HX \rightleftharpoons H_2X^+ + X^-$, and its magnitude must depend (aside from mobility corrections) on the product of the acidity and basicity constants of the liquid (6). Hence it is possible to infer that the basicity of hydrofluoric acid is greater, compared to its higher homologs, than would be expected from its relative acid strength in water. The stability of the fluoracidium salts of Hantzsch (32) supports this view. Many other interesting results may be expected from a combination of conductivity and E.M.F. methods of studying the acids.

In conclusion we may ask to what extent it is possible to reduce to a quantitative basis of comparison the data already accumulated and later to be secured on the variations of acid strength from solvent to solvent. If it were possible to establish a significant scale of relative acidities, so that one could say in an unequivocal manner that a certain solution in one solvent was more or less acid than a solution in another, whatever the acids present in either, the whole question would be reduced to a highly desirable order and simplicity. Such an outcome was apparently envisaged by Brönsted in his proposal of the acidity constant as

a general measure of acid strength and in his reference to a scale of "absolute (hydrogen ion) activity" (reference 6, page 292), although he speaks of the fact that "high and difficultly accessible boundary potentials will most likely obscure the results" (reference 6, page 351). If, however, the argument of Taylor and Guggenheim referred to above is accepted, and "the electric potential difference between two points is a conception without any physical significance unless the two points are in the same media," the notion of absolute acidity, as distinct from the relative activities of some particular acid in two different solutions, must be relegated to the limbo of seductive but meaningless fictions.

REFERENCES

- (1) BANDKE: Inauguraldissertation, Heidelberg (1898).
- (2) BISHOP, KITTREDGE AND HILDEBRAND: J. Am. Chem. Soc. 44, 135 (1922).
- (3) BJERRUM: III Nord. Kemistmötet, pp. 92-105, Finland (1928).
- (4) BJERRUM AND LARSSON: Z. physik. Chem. 127, 358-84 (1927).
- (5) BJERRUM, UNMACK AND ZECHMEISTER: Kgl. Danske Videnskab. Selskab. Math. fys. Medd. 5, No. 11 (1924).
- (6) BRÖNSTED: Chem. Reviews 5, 232-338 (1928).
- (7) BRÖNSTED: Ber. 61B, 2049-63 (1928).
- (8) BRÖNSTED: Z. angew. Chem. 43, 229-33 (1930).
- (9) CONANT AND BRAMANN: J. Am. Chem. Soc. 50, 2305 (1928).
- (10) CONANT AND HALL: J. Am. Chem. Soc. 49, 3062 (1927).
- (11) CONANT AND WERNER: J. Am. Chem. Soc. 52, 4436 (1930).
- (12) FREDENHAGEN: Z. anorg. allgem. Chem. 186, 1-37 (1930).
- (13) FREDENHAGEN: Z. physik. Chem. 141, 195-216 (1929).
- (14) FREDENHAGEN AND CADENBACH: Z. physik. Chem., Abt. A, 146, 245-80 (1930).
- (15) GERMAN: J. Am. Chem. Soc. 47, 2461 (1925).
- (16) GOLDSCHMIDT: Ann. 351, 108 (1907).
- (17) GOLDSCHMIDT: Z. physik. Chem. 99, 116-54 (1921).
- (18) GOLDSCHMIDT: Z. physik. Chem. 124, 23 (1926).
- (19) GOLDSCHMIDT AND AAS: Z. physik. Chem. 112, 423 (1924).
- (20) GOLDSCHMIDT AND BAKSCHT: Ann. 351, 108 (1907).
- (21) GOLDSCHMIDT AND MATHIESSEN: Z. physik. Chem., 119, 439-73 (1926).
- (22) GOLDSCHMIDT AND REINDERS: Ber. 29, 1366 (1899).
- (23) GOLDSCHMIDT AND SALCHER: Z. physik. Chem. 29, 89 (1899).
- (24) GROVE: J. Am. Chem. Soc. 52, 1404-7 (1930).
- (25) GUGGENHEIM: J. Phys. Chem. 34, 1758 (1930).
- (26) HALL: J. Am. Chem. Soc. 52, 5115 (1930).
- (27) HALL AND CONANT: J. Am. Chem. Soc. 49, 3047 (1927).
- (28) HALL AND WERNER: J. Am. Chem. Soc. 50, 2367 (1928).

- (29) HAMMETT: J. Am. Chem. Soc. 50, 2666 (1928).
(30) HAMMETT AND DIETZ: J. Am. Chem. Soc. 52, 4795 (1930).
(31) HANTZSCH: Z. Elektrochem. 29, 221 (1923).
(31a) HANTZSCH: Ber. 60B, 1933-50 (1927).
(31b) HANTZSCH: Z. physik. Chem. 134, 406-12 (1928).
(32) HANTZSCH: Ber. 63B, 1789-96 (1930).
(33) HANTZSCH AND VOIGT: Ber. 62B, 970-84 (1929).
(34) HANTZSCH AND WEISSBERGER: Z. physik. Chem. 125, 251-63 (1927).
(35) HLASKO AND KAMIENSKI: Roczniki Chem. 7, 6-22 (1927).
(36) International Critical Tables, Vol. VI, p. 142. McGraw-Hill Book Co., New York (1926).
(37) ISGARISCHEW AND PLETENEW: Z. Elektrochem. 36, 457 (1930).
(38) KILPATRICK: J. Phys. Chem. 34, 2180-6 (1930).
(39) KILPATRICK AND KILPATRICK: J. Am. Chem. Soc. 52, 1418 (1930).
(40) LARSSON: Inauguraldissertation, Lund (1924).
(41) LEWIS: Valence, p. 142. Chemical Catalog Company, New York (1923).
(42) MICHAELIS AND MIZUTANI: Z. physik. Chem. 116, 135-159 (1925).
(43) MIZUTANI: Z. physik. Chem. 116, 350 (1925).
(44) MIZUTANI: Z. physik. Chem. 118, 318-26, 327-41 (1925).
(45) PEDERSEN: J. Am. Chem. Soc. 53, 18-30 (1931).
(46) RABINOWITSCH: Z. physik. Chem., Abt. A, 147, 345-70 (1930).
(47) SCHREINER: Z. physik. Chem. 111, 419 (1924).
(48) SCHWARZENBACH: Helv. Chim. Acta 13, 870, 897 (1930).
(49) SIDGWICK: Electronic Theory of Valency, p. 116. Oxford (1928).
(50) TAYLOR: J. Phys. Chem. 31, 1478 (1927).
(51) WYNNE-JONES: J. Chem. Soc. 1930, 1064-71.
(52) ZINTL AND NEUMAYR: Ber. 63B, 237 (1930).

GENERAL REFERENCES

- HALL: J. Chem. Education 7, 782 (1930).
KOLTHOFF: Rec. trav. chim. 49, 401-14 (1930).

HYDROGEN FLUORIDE AND ITS SOLUTIONS

J. H. SIMONS

Department of Chemistry, Northwestern University, Evanston, Illinois

Received January 12, 1931

The study of hydrogen fluoride is beset with difficulties and dangers, but the valuable results obtained compensate for the obstacles that must be overcome. A more complete knowledge of hydrogen fluoride should add much to chemistry in general, for it is in many respects a unique compound. For instance, it has the ability to form addition compounds, and has the highest heat of formation of the hydrogen halides. Compared with the other hydrogen halides it is a moderately weak acid in aqueous solution, and its boiling and freezing points are not in agreement with the trend of these for the other hydrogen halides. The structures of the molecular species, H_6F_6 and HF_2^- , which have been reported for hydrogen fluoride, are a challenge to our theories of molecular structure and must be explained by any complete picture of chemical compounds.

Solutions in liquid hydrogen fluoride have been studied very little until quite recently. It is probably because the technique of handling anhydrous hydrogen fluoride is different from that of working with other substances that it has been almost neglected by chemists. Glass vessels are not suitable containers for it, and our technique of constructing apparatus entirely of metal and operating it without being able to observe visually the processes that are taking place has not been highly developed.

Hydrogen fluoride was first obtained by Margraff (1) in 1768, but it was Sheele (2) who in 1771 definitely characterized it. A pure and highly concentrated aqueous solution was prepared by Thénard and Gay-Lussac (3) in 1809. Davy (4) in 1813 and the years following worked with hydrogen fluoride and proved that it contained no oxygen. He experimented with passing an

electric current through the liquid and also the gas. He actually obtained at the end of the electrolysis a liquid which would not conduct the current, indicating that he had prepared the liquid in a high state of purity. It is reported that he ceased his experiments with this material when he found that they were injurious to his health. Fremy (5) in 1856 was the first to make anhydrous hydrogen fluoride and by the method that is still used, that is, by heating purified and dried potassium hydrogen fluoride. Gore (6) in 1869 also studied hydrogen fluoride. He showed that it would not conduct the electric current, but that it became a conductor when a small amount of water was present. The early history of hydrogen fluoride is perhaps ended with the work of Moissan (7). He prepared a liquid of high purity, found that potassium fluoride was soluble in it to give a conducting solution, and prepared fluorine by the electrolysis of this solution.

THE PREPARATION OF ANHYDROUS HYDROGEN FLUORIDE

The preparation of hydrogen fluoride by the treatment of calcium fluoride with sulfuric acid does not give a material that is pure or anhydrous (8). The difficulties in the method used by Fremy lie chiefly in drying the potassium hydrogen fluoride. Moissan (9) describes the method he used for doing this as follows:

In order to obtain pure anhydrous hydrofluoric acid, one begins by preparing the fluohydrate of potassium fluoride, taking all the precautions indicated by Fremy. Having obtained this salt in a state of purity, it is dried over the water bath at a temperature of 100°C. and afterwards the capsule containing it is placed in vacuo in presence of sulfuric acid and of caustic potash fused in a silver crucible. The acid and potash are replaced every morning during fifteen days, and the vacuum in the bell jar is always maintained to a pressure of about one centimeter of mercury.

During this desiccation, it is necessary to pulverize the salt from time to time in an iron mortar, in order to expose fresh surfaces. When the fluohydrate no longer contains water, it falls into a fine powder and can then be used for the preparation of hydrofluoric acid. It is to be noted that well-made fluohydrate of potassium fluoride is much less deliquescent than the normal salt.

When the fluohydrate is thoroughly dry, it is quickly transferred to a platinum alembic, which has been dried at a red heat a little while before. It is heated gently for an hour or an hour and a half, in order that the decomposition may begin slowly, and the first portions of the hydrofluoric acid formed, which may contain traces of water remaining in the salt are rejected. . . .

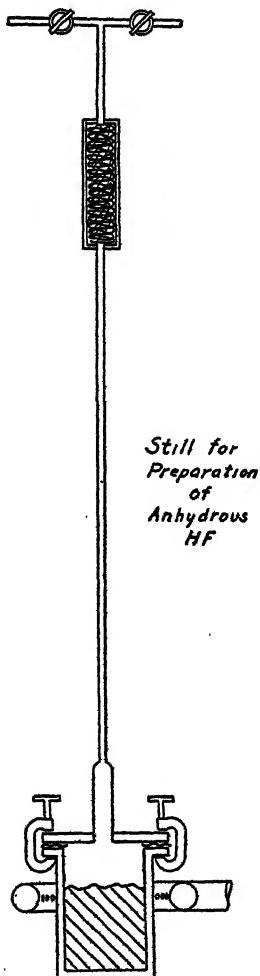


FIG. 1. STILL FOR THE PREPARATION OF ANHYDROUS HYDROGEN FLUORIDE

Fredenhagen and Cadenbach (10) have shown that the acid salt loses hydrogen fluoride gradually until at a temperature of 504°, when about 30 per cent has been lost, the temperature remains constant, and the remainder of the gas is liberated. They have also shown that any small amount of water remaining in the salt comes off with the first portions of hydrogen fluoride distilled at the higher temperatures. They have been able to reduce the residual water in the almost anhydrous liquid by repeated distillations.

The tedious drying of the salt and the equally difficult repeated distillations of the liquid have been eliminated in the method of Simons (11). He completely dehydrates the salt by electrolysis in the fused state. The distillation of the resulting material produces a product that must be completely anhydrous. Fluorine is produced smoothly and in quantity only after all the water has been removed from the salt. This enables one to determine easily when it is completely dry. As the molten salt has a tendency to foam during the distillation, Simons has preferred to use the still shown in figure 1 heated by means of a ring burner. As the electrolysis is carried on in the base of the still, there is no necessity of transferring the material and no danger of absorbing water during such a process.

THE TECHNIQUE OF HANDLING HYDROGEN FLUORIDE

Improvements in the technique of handling anhydrous hydrogen fluoride have been very important in its study. As the anhydrous liquid absorbs water with great vigor, all moisture must be excluded. The liquid acts as a good dehydrating agent, hence any material from which water may be extracted, such as Bakelite, many organic materials, etc., must be avoided. Siliceous materials are of course reacted upon, and although the action on quartz is slow, it is, nevertheless, appreciable. Fredenhagen and Cadenbach (12) were, however, able to determine qualitative solubilities in a fused quartz vessel because of the slowness of the action. The reaction or solubility of so many of the inorganic compounds makes them inadequate for use as containers. Even the slight solubility of calcium fluoride would pre-

vent fluorite from being used for quantitative experiments. Sulfur has been used successfully for the insulator separating the electrodes in conductivity experiments by Fredenhagen and Cadenbach. Apparatus constructed for the study of anhydrous hydrogen fluoride should in general be made entirely of metal—preferably of platinum, gold, or silver. Copper may be used, but apparatus made of it should be kept filled with dry nitrogen. Any oxide or other coatings on the metal should be carefully removed, and the apparatus scrupulously dried. Permanent joints can be made with silver solder; others can be made by some sort of metal to metal screw or pressure connections. The apparatus should be sealed directly to the vessel in which the anhydrous liquid is prepared, so that the liquid can be distilled directly into the apparatus.

CHEMICAL PROPERTIES

Although hydrogen fluoride does not react appreciably with most of the elementary metals, it reacts with sodium in the same manner that water does. It does not react with magnesium. With the chlorides, bromides, and iodides of the alkali metals and of the alkaline earth metals, and with ferrous chloride, manganeseous chloride, and cerous chloride, it liberates the hydrogen halide and forms the fluoride. It reacts in the cold with the hydroxides of the metals and with many of the oxides, such as those of magnesium, calcium, strontium, barium, silver and also with lead monoxide. It reacts slowly with aluminum oxide, silicon dioxide, and cupric oxide. With potassium and barium chlorates, it liberates chlorine dioxide. It liberates hydrogen cyanide from potassium cyanide, and silicon tetrafluoride from potassium fluosilicate. It unites with water with considerable evolution of heat, and freezing point data indicate the crystalline compounds $H_2O \cdot HF$ (13), $H_2O \cdot 2HF$, and $H_2O \cdot 4HF$ (29). With the alkaline fluorides it forms a number of crystalline compounds, as for example $KFHF$, $KF(HF)_2$, $KF(HF)_3$, etc. (14). In these compounds it is probably analogous to water of crystallization. It is a good dehydrating agent and will remove water from many substances. It reacts with acetic anhydride to produce acetyl

fluoride and will react with the aliphatic alcohols at high temperatures to produce the fluorides.

PHYSICAL PROPERTIES

The boiling point (15) of hydrogen fluoride is 19.5° ; freezing point (16), -83° ; heat of fusion, 1090 calories per mole of HF; heat of vaporization (11), 6020 calories per apparent molecular weight in the gas phase; heat of formation (16), 63000 calories per mole; specific conductivity (10), less than 1.4×10^{-5} ; dielectric constant (17) 174.8 at -73° , 173.2 at -70° , 134.2 at -42° , 110.6 at -27°

TABLE 1

Comparison of the properties of hydrogen fluoride with those of related compounds

	FREEZING POINT °C.	BOILING POINT °C.	MOLAR HEAT OF FUSION calories	MOLAR HEAT OF VAPORIZATION calories	DIELECTRIC CONSTANT
HF.....	-83	19.5	1.09×10^3	6.02×10^3	83.5 (0°)
HCl.....	-114	-85.8	0.50×10^3	3.6×10^3	4.60 (27.7°)
HBr.....	-86	-67.1	0.62×10^3	4.0×10^3	3.82 (24.7°)
HI.....	-53.6	-36.0	0.72×10^3	4.4×10^3	2.9 (21.7°)
H ₂ O.....	0	100	1.34×10^3	9.72×10^3	80 (20°)
H ₂ S.....	-85.5	-61.8			5.75 (10°)
H ₂ Se.....	-64	-42			
H ₂ Te.....	-48	-1.8			
NH ₃	-77	-38.5	1.84×10^3	5.6×10^3	14.9 (24.5°)
PH ₃	-132.5	-86.4			2.71 (-25°)
AsH ₃	-113.5	-54.8			2.05 (15°)
SbH ₃	-91.5	-18			2.58 (-50°)
HCN.....	-13.8	26.5		5.7×10^3	95 (21°)

and 83.6 at 0° . Its vapor pressure has been shown to fit the equation

$$\log P = 7.37 - \frac{1315}{T},$$

where P is the pressure in millimeters of mercury and T is the temperature in degrees K.

A comparison of the physical properties of hydrogen fluoride with those of the other hydrogen halides and with water, ammonia, and hydrogen cyanide is shown in table 1. It is apparent that hydrogen fluoride does not follow the properties of the other

hydrogen halides in the expected manner; it shows greater similarity to water and to hydrogen cyanide, than it does to the halides. Its melting point, freezing point, heat of vaporization, and heat of fusion, instead of being lower than those of hydrogen chloride, are higher. It is interesting that the relation between the properties of water and those of hydrogen sulfide, hydrogen selenide, and hydrogen telluride, and the relation between the properties of ammonia and those of phosphine, arsine, and stibine is the same as the relation between the properties of hydrogen fluoride and those of the other hydrogen halides.

MOLECULAR STRUCTURE

In order to account for the peculiar properties of water, it has been assumed that it is polymerized or associated in the liquid state. Some investigators have assumed definite molecular species, such as $(H_2O)_2$, $(H_2O)_3$, etc. and have considered them to exist in equilibrium with one another. Others have considered chain molecules of indefinite and indeterminable size from which the single molecules are continually being gained and lost. One might also assume that instead of definite polymers being formed, the simple molecules exert unusually large attractive forces upon one another. This must be true in any case, as seen by the large electric moment of the water molecule. Polymerization has also been assumed for hydrogen fluoride in the liquid state in order to account for its peculiar properties.

Its structure is, therefore, a matter of considerable interest. The formula H_2F_2 is generally seen in text-books, despite the fact that investigators are now in agreement that this molecular species has never been found. This formula has come into our literature, because at warm room temperature and atmospheric pressure the density of the gas corresponds to the density which the formula H_2F_2 would give. An early worker, Mallet (18), made this determination, assigned the formula, and it has been retained. A variation of temperature changes the apparent molecular weight considerably, as shown by Thorpe and Hambly (19); in fact, just above the boiling point the density is much greater than H_2F_2 would give. A variation of pressure also

varies the apparent molecular weight. These results indicate that if H_2F_2 exists at all, it is certainly not the only or even the major molecular species. There is another argument that has been used in favor of H_2F_2 , and that is that the salts KFHF and NaFHF exist as crystalline compounds. This is of course a very weak argument, for the crystalline compounds $KF(HF)_2$ and $KF(HF)_3$ also exist. This is the same reasoning one would use in assigning the formula for water on the basis of the number of molecules of H_2O that are retained as water of crystallization in a particular compound. There is fairly good evidence for the existence of the HF_2^- ion in aqueous solutions, but this does not indicate the existence of H_2F_2 . In fact, the experimental data give no indication of this molecular species, although it has been looked for.

Simons and Hildebrand (20) were able to obtain apparent molecular weights for gaseous hydrogen fluoride as high as 87.4, showing that there must be a molecular species containing more than four HF molecules. They did this by comparing the vapor pressure determined by the static method by Simons (11) with that determined by the dynamic method. In the dynamic method of determining vapor pressure, the value of the molecular weight must be assumed in order to calculate the number of moles taken through the apparatus per mole of the carrier gas. When the vapor pressure is known, the molecular weight becomes the unknown factor and can be calculated from the experimental data. By assuming an equilibrium between two molecular species in the gas phase according to the equation

$$n \text{ HF} = (\text{HF})_n$$

with the equilibrium constant K expressed in terms of the partial pressures of these species p and p_n , by the equation $K = \frac{p_n}{p^n}$, they have been able to account for their determinations of apparent molecular weight which extend from -39° to $+16^\circ$ over which range the vapor pressure of HF changes from 56.2 mm. to 661 mm. of Hg, and also the extensive vapor density determinations of Thorpe and Hambly (19), which at constant pressure

extend from 26.5° to 88.2° and at constant temperature extend from 353 mm. up to atmospheric pressure. Thorpe and Hambly used two methods of reducing the pressure: one by reducing the total pressure in the apparatus, and the other by diluting the hydrogen fluoride with air. These results checked, and they carried out the constant temperature experiments at two different temperatures. Various values of n were tried, and an agreement

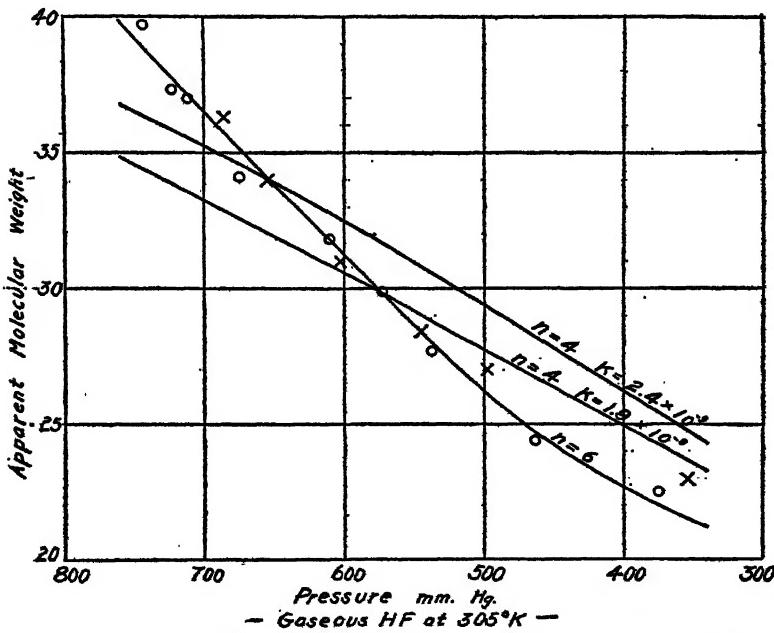


FIG. 2. VARIATION OF APPARENT MOLECULAR WEIGHT OF GASEOUS HYDROGEN FLUORIDE WITH PRESSURE

with the experimental data was found with $n = 6$. Figure 2 shows the good agreement of the theoretical and experimental values for apparent molecular weight, as the pressure is varied with the temperature constant at 305°K . At this temperature the equilibrium constant equals 2.91×10^{-15} . By treating the equilibrium constant in the usual way, the effect of temperature upon it is given by the equation:

$$\log K = \frac{-\Delta H}{4.579 T} + C.$$

When $\Delta H = -40,000$ calories and $C = -43,145$, the apparent molecular weight calculated from K in the above equation fits the experimentally determined values taken at constant pressure and varying temperature, as shown in figure 3 where the smooth curve represents the calculated values. Of course the test of this theory comes in comparing the calculated values with those obtained at low temperatures and at the saturation pressure. Figure 3 shows that this agreement is good.

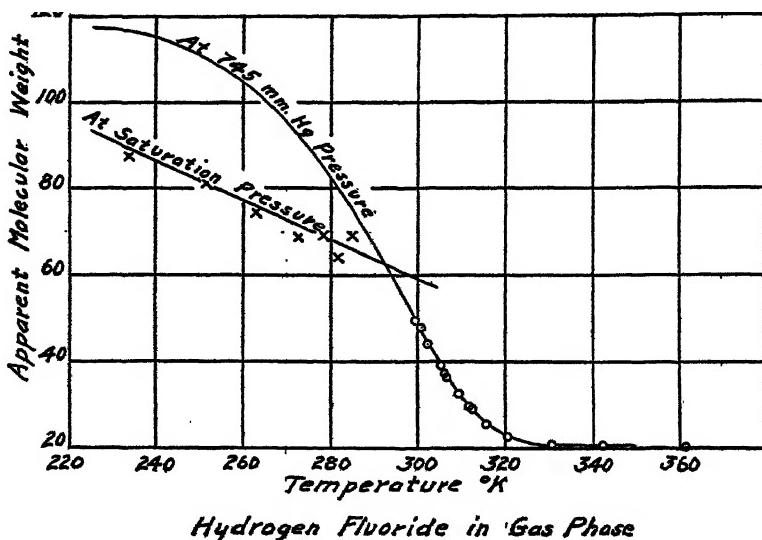


FIG. 3. VARIATION OF APPARENT MOLECULAR WEIGHT OF GASEOUS HYDROGEN FLUORIDE WITH TEMPERATURE

As the simple assumption of the equilibrium $6 \text{ HF} = \text{H}_6\text{F}_6$ agrees well with molecular weight determinations taken over a wide range of temperature and pressure, the evidence in favor of the molecular species H_6F_6 in the gas phase is very good. There is no indication of H_2F_2 , and if it is present, it must be in very small concentration. The molecular structure of the liquid phase of hydrogen fluoride is not established; but it is interesting to note that the heat of association, -40,000 calories for six moles of HF or 6,670 calories per mole of HF, is close to its heat of vaporization.

SOLUBILITY OF SUBSTANCES IN ANHYDROUS LIQUID HYDROGEN
FLUORIDE

Gore and Moissan were interested in solutions in liquid hydrogen fluoride, but neither of them made any systematic study of hydrogen fluoride as a solvent. In a footnote in one of his papers, Franklin (21) reports the qualitative solubility of a number of substances, chiefly inorganic salts, in liquid hydrogen fluoride. Fredenhagen has made a systematic study of the qualitative solubility of a large number of inorganic substances. Table 2 gives a summary of the solubilities of inorganic substances in liquid anhydrous hydrogen fluoride. In the table are given the quantitative solubilities, where they are known, in terms of grams of the solute in cubic centimeters of the solution. In cases where a reaction occurs and a gaseous product is formed, the formula of the gas is given in parentheses. The hydroxides and oxides that react produce water, which remains in the solution. It is interesting to note that the solubility of the fluorides of the alkali metals and also of the alkaline earth metals increases with the molecular weight. This is directly analogous to the solubilities of the hydroxides of the same elements in water.

Hydrogen fluoride is found to be a polar solvent very similar to water, as one would expect from the fact that its dielectric constant is near that of water. Some of this solubility data is contrary to what one might expect. An example of this is the insolubility of silicon tetrafluoride. It apparently does not combine with the solvent to form hydrofluosilicic acid. The insolubility of the other hydrogen halides does not follow the analogy with water. It is interesting to note that potassium cyanide evolves hydrogen cyanide, but that mercuric cyanide does not, and that mercuric cyanide is soluble, but that mercuric fluoride is not. The most surprising result is that hydrogen cyanide is not soluble, for hydrogen cyanide is a compound which appears to be very similar to hydrogen fluoride, having a high dielectric constant and a boiling point not greatly different. One would expect these liquids to be miscible.

TABLE 2
Solubility of inorganic substances in hydrogen fluoride

VARYING SOLVENT	SOLUBILITY SOLVENT	NOT APPRECIABLY SOLUBLE	SOLUBLE WITH REACTION	REACT BUT PRODUCT INSOLUBLE		INSOLUBLE AND UNREACTIVE
				ALKALI HALIDES AND ALKALINE EARTH HALIDES DISSOLVE TO FORM HYDROGEN HALIDES	KCN(HCN)	
H ₂ O	MgF ₂ , CaF ₂ , SrF ₂ , BaF ₂ , NaF, KF (36 per 100 at 0°), RbF, CsF, TlF, AgF (33 per 100 at -15°), Hg(CN) ₂ , KNO ₃ , NaNO ₃ , AgNO ₃ , K ₂ SO ₄ , Na ₂ SO ₄	AlF ₃ , ZnF ₂ , FeF ₃ , PbF ₂ , CuF ₂ , HgF ₂ , HCl, H ₂ S, CO, CO ₂ , HgI ² , HBr, HI, SiF ₄ , Cu(NO ₃) ₂ , Bi(NO ₃) ₃ , Pb(NO ₃) ₂ , Co(NO ₃) ₂ , ZnSO ₄ , CdSO ₄ , CuSO ₄ , Ag ₂ SO ₄	AlCl ₃ (HCl), FeCl ₃ (HCl), MnCl ₃ (HCl), CeCl ₃ (HCl), MgO, CaO, SrO, BaO, PbO, BaO ₂ , Al ₂ O ₃ , CuO	AlCl ₃ , FeCl ₃ , SnCl ₃ , NiCl ₃ , CdCl ₃ , CuCl ₂ , HgI ₂ , AgCl, AgBr, AgI, HgO, PbO ₂ , MnO ₂ , SnO ₂ , Cr ₂ O ₃ , WO ₃ , Mn ₂ O ₃	ZnCl ₂ , SnCl ₂ , NiCl ₂ , CdCl ₂ , CuCl ₂ , HgI ₂ , AgCl, AgBr, AgI, HgO, PbO ₂ , MnO ₂ , SnO ₂ , Cr ₂ O ₃ , WO ₃ , Mn ₂ O ₃	
NH ₄ F						
LiF (2.6 per 100 at 18°)						

CONDUCTANCE OF SOLUTIONS IN ANHYDROUS HYDROGEN FLUORIDE

Gore found that water formed a conducting solution, when dissolved in hydrogen fluoride, and Moissan (7) found that potassium fluoride did likewise. It has remained for Fredenhagen and Cadenbach (12) to determine the equivalent conductivities of a

TABLE 3
Conductivity of substances in solution in hydrogen fluoride

Normality.....	0.5	0.24	0.115	0.055	0.026	0.013
Dilution.....	2.0	4.2	8.7	18	38	79
Equivalent conductivity at -15°						
H ₂ O.....	121	153	183	210	228	242
KF.....	172	203	225	241	251	255
AgF.....	174	201	222	238	247	255
KNO ₃	360	429	482	558	610	622
AgNO ₃	390	470				
H ₂ SO ₄		151	184	209	232	245
½K ₂ SO ₄	171	211	244	267	276	277
CH ₃ OH.....	139	164	200	225	243	251
C ₂ H ₅ OH.....	166	180	210	233	246	256
C ₃ H ₇ OH.....	142	163	196	215	231	236
C ₆ H ₅ OH.....	128	158	186	205	222	224
HCOOH.....	77	102	132	166	198	237
CH ₃ COOH.....	148	175	205	227	241	260
CCl ₃ COOH.....	9	11	11			
½(COOH) ₂	94	117	137	161		
C ₆ H ₅ COOH.....	176	181	190	200	226	243
(CH ₃ CO) ₂ O.....	154	180	208	226	244	
CH ₃ COOK.....	325	367				
CH ₃ COCH ₃	159	182	216	238	252	273
(C ₂ H ₅) ₂ O.....	179	190	216	269	293	310
Glucose.....	114	165	208	247	279	310
C ₆ H ₅ OH.....	17	23	31	44	52	59
C ₆ H ₅ (NO ₂) ₂ OH.....	only 0.2 per 100 soluble: conductivity less than for phenol					

number of solutes over a range of dilution. A summary of their measurements is given in table 3, and figure 4 shows these data graphically. Potassium fluoride and silver fluoride give very nearly the same conductivities, and they are apparently completely ionized in the more dilute solutions. The results for

potassium nitrate, silver nitrate, and potassium acetate are surprising, for these salts are binary electrolytes in aqueous solution, but in this solvent they apparently produce twice the number of ions that the binary salts potassium fluoride and silver fluoride produce. In order to account for this result, Fredenhagen and Cadenbach have first considered the ionization of

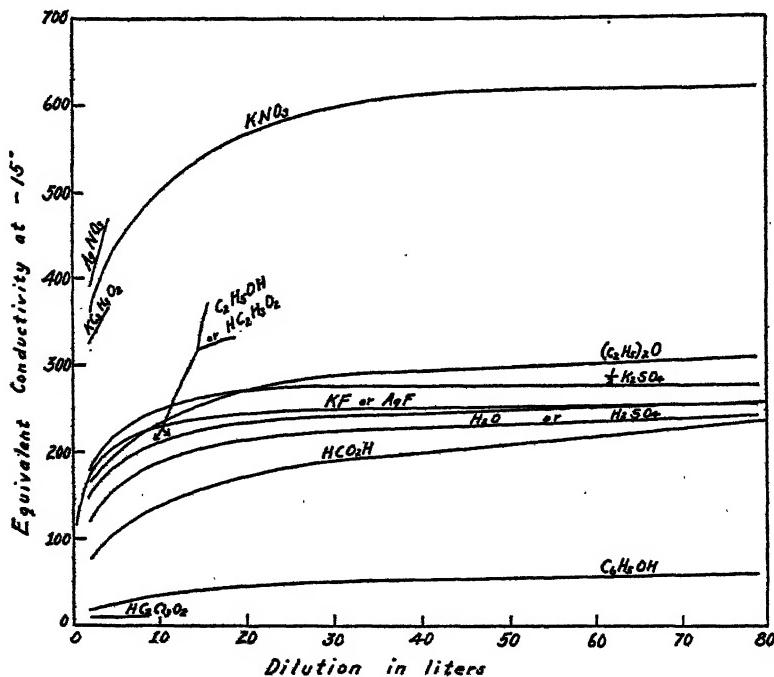
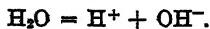
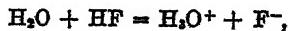


FIG. 4. CONDUCTIVITIES IN LIQUID ANHYDROUS HYDROGEN FLUORIDE

water. The data show this as a binary electrolyte, the ionization of which could be represented as



As hydrogen fluoride is an acid material, it may serve as donor of protons to the water according to the equation

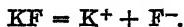


which would produce the same number of ions and so give a similar equivalent conductivity. In order to distinguish between these two possibilities, an equal molal mixture of water and potassium fluoride was dissolved in hydrogen fluoride and the conductivities determined. The results given in table 4 show that the conductivity of the mixture is approximately equal to the sum of the conductivities of the two solutes taken separately. This decides rather conclusively for the second mode of ionization of water for

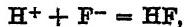
TABLE 4
Conductivities of KF and H₂O singly and together in HF at -15°C.

CONCENTRATION moles per liter	KF AND H ₂ O TOGETHER	KF	H ₂ O	SUM OF KF AND H ₂ O TAKEN SINGLY
0.5	0.1126	0.0863	0.0606	0.1469
0.24	0.0685	0.0488	0.0367	0.0855
0.115	0.0396	0.0258	0.0214	0.0473
0.055	0.0224	0.0133	0.0115	0.0248
0.026	0.0124	0.00663	0.00600	0.0126
0.013	0.00620	0.00321	0.00305	0.00626

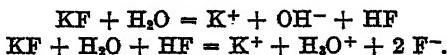
the following reason. The potassium fluoride ionizes according to the equation



If the water ionizes to produce hydrogen ions, these would react to produce the unionized solvent with the fluoride ion

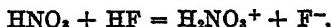


and instead of four ions per mole each of water and potassium fluoride, there would result only two, as the following equations show:

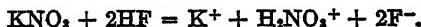


The first equation is excluded on the basis of chemical knowledge, for potassium hydroxide reacts vigorously with anhydrous hydrogen fluoride to produce potassium fluoride and water, whereas the first equation shown immediately above represents the reverse of this reaction.

The high conductivity of potassium nitrate and silver nitrate is explained in a similar manner. The ionization of nitric acid in hydrogen fluoride is assumed to be according to the equation



and that of potassium nitrate

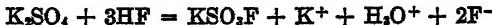


This is, of course, equivalent to saying that nitric acid acts as an acceptor for protons from hydrogen fluoride or that it is basic in relation to it.

The conductivities of sulfuric acid are practically identical with those for water, and this is accounted for by a reaction to produce water.



The analogous reaction for potassium sulfate is



However, a small ionization of KSO_4F must be assumed.

The conductivity of acetic acid could be accounted for in two ways. The first would be a reaction with hydrogen fluoride to form acetyl fluoride and water,



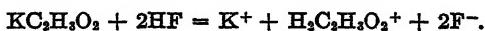
and in the second, the acetic acid molecule would act as an acceptor for the proton from the hydrogen fluoride



In the first case, we should expect the conductivity to be exactly the same as for water, as was found for sulfuric acid. This is not true. We also know that acetyl fluoride does not ionize in this solution, because acetic anhydride gives conductivities practically identical with those of acetic acid. We know that acetic anhydride reacts to form acetyl fluoride and acetic acid according to the equation



This permits us to explain the conductivity of potassium acetate by the reaction



The acceptor theory to account for the conductivities of the organic acids allows us to explain the apparently low ionization of trichloroacetic acid. In aqueous solution, it is a stronger acid than acetic acid; this fact shows it to be a stronger donor and therefore a weaker acceptor. Being a weaker acceptor, it would have less tendency to take the proton from the hydrogen fluoride than acetic acid. The same reasoning accounts for the higher conductivity of benzoic acid as compared with acetic acid, for benzoic acid is a weaker acid in aqueous solution than acetic acid. Oxalic acid is a stronger acid in aqueous solution than acetic acid and in hydrogen fluoride shows a lower conductivity; this fact agrees with the theory, as oxalic acid would be a poorer acceptor than acetic acid. The same reasoning can be applied to formic acid.

The alcohols act as binary electrolytes in hydrogen fluoride solution. This may be accounted for in two ways: either they react to form a fluoride and water, or they act as acceptors for protons. The latter explanation seems to correspond more closely to the truth, as can be seen in the case of ethyl alcohol. If the former explanation held, we should expect the conductivity to be the same as that for water, whereas it is appreciably higher. In addition, ethyl alcohol has been shown not to react appreciably with hydrogen fluoride at room temperatures. Meslans and Girardet (22) found the reaction to begin only at 140°, and Fredenhagen (23) has been able to recover the alcohol from its solution in hydrogen fluoride without obtaining ethyl fluoride. Phenol is an alcohol which produces considerable hydrogen ions in aqueous solution. It is, therefore, a better donor of protons than ethyl alcohol and would be expected to be a poorer acceptor for them in hydrogen fluoride solution and so give a lower conductivity, which it does. Picric acid gives a lower conductivity than phenol, as we should expect from the fact that it is a stronger acid and therefore a weaker base.

The high conductivities of acetone and ethyl ether can also be accounted for on the basis that they act as acceptors for hydrogen ions in hydrogen fluoride. This agrees well with our knowledge of the structure and properties of these compounds. They seem to act as strong bases in these solutions, and if the conductivity measurements are significant, begin to accept the second proton at high dilution.

The insolubility of the other hydrogen halides can be well explained by this theory. They have much lower boiling points and low dielectric constants. Unless they ionized in the solution or reacted with the solvent, we should not expect them, on the

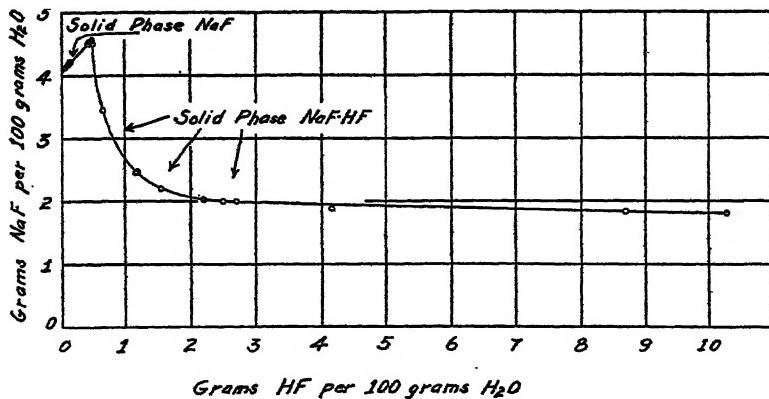
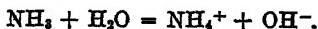


FIG. 5. SOLUBILITY OF SODIUM FLUORIDE IN AQUEOUS HYDROFLUORIC ACID

basis of our ordinary laws of solubility, to be soluble. As they are themselves donors of protons, with little tendency to act as acceptors, they could not react with hydrogen fluoride, which cannot act as an acceptor. They apparently cannot ionize in the absence of an acceptor, for they do not ionize when in the pure liquid state.

A comparison of the three liquids—ammonia, water, and hydrogen fluoride—shows that ammonia is more basic than water, and water more basic than hydrogen fluoride. Ammonia reacts with water in a manner similar to the reaction of water with hydrogen fluoride, namely



Water acts as a donor to ammonia but as an acceptor to hydrogen fluoride. A strongly acid compound like hydrogen chloride acts as a donor to both ammonia and water but not to hydrogen fluoride, while a weaker acid compound like acetic acid acts as a donor to both ammonia and water but as an acceptor to hydrogen fluoride. The properties of a polar solvent are seen to depend not only upon the dielectric constant, but also upon the tendency to act as acceptor or donor of protons. Many of the properties of water are and have been explained by its ability to act in either capacity. Hydrogen fluoride apparently acts chiefly as a donor.

THE SYSTEM BENZENE—HYDROGEN FLUORIDE

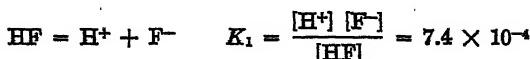
The author (30) has made a study of the system benzene-hydrogen fluoride in the region of a very low proportion of hydrogen fluoride. The solubility of hydrogen fluoride in the gas phase was measured in benzene over a range of both temperature and partial pressure of hydrogen fluoride over the solution. Figure 5 shows the partial pressure of hydrogen fluoride in the gas phase plotted against its mole fraction in the solution. The straight lines in the graph represent the "ideal" solution, and so it is seen that the deviation from ideality is very great. This represents an unusual type of solution with a large positive deviation from Raoult's law at the higher concentrations and a negative deviation in the more dilute solution. The positive deviation is to be expected, because of the great difference between the dielectric constants of the two compounds, but the negative deviation is a surprising result.

A possible explanation of this phenomenon can be given by a consideration of the electric field surrounding the highly polar hydrogen fluoride molecule. When these molecules are in sufficient concentration to exert an influence upon one another, they will be mutually attracted and so tend to be removed from the solution. This explains the positive deviation from Raoult's law at high concentration. When the concentration is so low that the distances between the molecules of hydrogen fluoride are great enough to make the mutual attraction small, then the electric field around each hydrogen fluoride molecule will attract

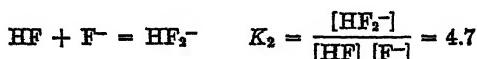
even the slightly polar benzene molecules and so cause the vapor pressure of hydrogen fluoride from the solution to be less than would be given by Raoult's law.

AQUEOUS SOLUTIONS OF HYDROGEN FLUORIDE

Aqueous solutions of hydrogen fluoride have been more extensively studied than has the pure liquid. After a number of authors had favored either HF or H_2F_2 as the formula for the undissociated molecules in aqueous solutions, Pick (24) suggested that the properties of hydrogen fluoride could be accounted for quantitatively as well as qualitatively by the assumption of the HF_2^- ion and the absence of the H_2F_2 molecules. He explained the conductivity of dilute hydrofluoric acid solutions very well by this means. In a series of papers by Hudleston and his co-workers, it has been shown that this assumption would account for the experimental data obtained in transference (25), hydrogen ion activity (26), and freezing point lowering experiments (27), and also the solubility of sodium fluoride (28) in dilute aqueous solutions with a varying concentration of hydrogen fluoride. By using the assumption of the two equilibria



and



they have been able to explain this entire mass of data. Their work on freezing point lowering in particular gives no indication of H_2F_2 but rather shows that, if it is present, it must be in extremely small concentration. Of course the existence of HF_2^- is no indication of H_2F_2 any more than the existence of NH_4^+ is an indication of N_2H_6 .

The solubility of sodium fluoride in aqueous solution increases markedly as hydrogen fluoride is added to the solution, and then decreases as shown in figure 6. Jehu and Hudleston (28) have explained this with the above equilibria. As the hydrogen fluor-

ide is added, it reacts with the fluoride ion and so increases the solubility of the sodium fluoride, the ionization of the hydrogen fluoride being small. When a sufficient concentration of HF_2^- is reached, the addition of more hydrogen fluoride increases it above the solubility product of $\text{Na}^+ + \text{HF}_2^-$, and so reduces the solubility of sodium fluoride in the solution.

Some unfinished experiments, which the author is conducting on the electrometric titration of aqueous solutions of hydrogen

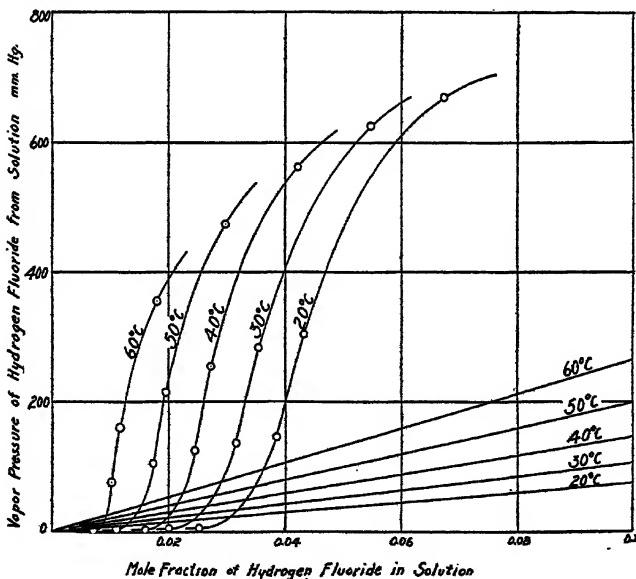
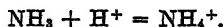


FIG. 6. HYDROGEN FLUORIDE SOLUTION IN BENZENE

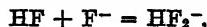
fluoride with solutions of potassium fluoride and ammonium fluoride, indicate that hydrofluoric acid is not a dibasic acid. This means that the ion HF_2^- does not ionize to produce hydrogen ion and the ion F_2^{--} . This may mean that in the ion HF_2^- the connection is not made through the two fluorine atoms, but through the hydrogen after the manner of the hydrogen bond of Lewis.

The knowledge of the HF_2^- ion adds to our comparison of the

three polar liquids—ammonia, water, and hydrogen fluoride. Comparing the other two in solution in water, we find that ammonia is basic and so adds the positive hydrogen ion



Hydrogen fluoride, being acid, adds the negative fluoride ion,



in an analogous reaction. It probably adds the fluoride ion in preference to the hydroxyl ion, as the fluoride ion is the more plentiful in aqueous hydrofluoric acid solutions. As the modern concept of a base is that it is not only a substance which will produce hydroxyl ions but also one that acts as an acceptor for hydrogen ions, so an acid can be considered not only as a substance which acts as a donor of hydrogen ions, but also as a substance which acts as an acceptor of its own negative ion. The strong tendency of hydrogen fluoride to act as an acid on this basis probably accounts for its being an apparently weak acid in aqueous solution. The fluoride ion in anhydrous hydrogen fluoride solutions is probably also solvated.

REFERENCES

- (1) MARGRAFF: *Memoires de Berlin*, p. 3 (1768).
- (2) SHEELE: *Svens. vetens. Acad.*, p. 120 (1771).
- (3) THÉNARD AND GAY-LUSSAC: *Ann. chim. phys.* (1) **59**, 204 (1809).
- (4) DAVY: *Phil. Trans.* **103**, 263 (1813).
- (5) FREMY: *Ann. chim. phys.* (8) **47**, 5 (1856).
- (6) GORE: *Phil. Trans.* **159**, 173 (1869).
- (7) MOISSAN: *Le Fluor et ses Composes*. G. Steinheil, Paris (1900).
- (8) RUFF: *Die Chemie des Fluors*, p. 22. Julius Springer, Berlin (1920).
- (9) MOISSAN: *Annual Report Smithsonian Institute* **1**, 256.
- (10) FREDENHAGEN AND CADENBACH: *Z. anorg. allgem. Chem.* **178**, 289 (1929).
- (11) SIMONS: *J. Am. Chem. Soc.* **46**, 2179 (1924).
- (12) FREDENHAGEN AND CADENBACH: *Z. physik. Chem.*, Abt. A, **146**, 245 (1930).
- (13) METZNER: *Compt. rend.* **119**, 682 (1894).
- (14) FREMY: *Ann. chim. phys.* (8) **47**, 5 (1856).
- MOISSAN: *Compt. rend.* **108**, 547 (1888).
- EGGELING AND MEYER: *Z. anorg. chem.* **46**, 174 (1905).
- (15) GORE: *J. Chem. Soc.* **22**, 368 (1869).
- (16) V. WARTENBERG: *Z. anorg. allgem. Chem.* **151**, 326 (1926).
- (17) FREDENHAGEN AND DAHMLOS: *Z. anorg. allgem. Chem.* **178**, 272 (1928).

- (18) MALLET: Am. Chem. J. **3**, 189 (1881).
- (19) THORPE AND HAMBLY: J. Chem. Soc. **53**, 765 (1883).
- (20) SIMONS AND HILDEBRAND: J. Am. Chem. Soc. **46**, 2183 (1924).
- (21) FRANKLIN: Z. anorg. allgem. Chem. **46**, 2 (1905).
- (22) MESLAMS AND GIRARDET: Compt. rend. **115**, 1080 (1892).
- (23) FREDENHAGEN: Z. physik. Chem. **128**, 15 (1927).
- (24) PICK: Nernst's "Festschrift" (1912), p. 360.
- (25) DAVIES AND HUDLESTON: J. Chem. Soc. **125**, 260 (1924).
- (26) WINNE-JONES AND HUDLESTON: J. Chem. Soc. **125**, 1081 (1924).
- (27) ANTHONY AND HUDLESTON: J. Chem. Soc. **127**, 1122 (1925).
- (28) JEHU AND HUDLESTON: J. Chem. Soc. **125**, 1451 (1924).
- (29) CADY AND HILDEBRAND: J. Am. Chem. Soc. **52**, 3843 (1930).
- (30) SIMONS: J. Am. Chem. Soc. **53**, 83 (1931).

LIQUID HYDROGEN SULFIDE AS A REACTION MEDIUM

JOHN A. WILKINSON

Department of Chemistry, Iowa State College, Ames

Received January 12, 1931

The amount of work which has been done with liquid hydrogen sulfide as a reaction medium is small, due chiefly to the fact that while hydrogen sulfide is an inorganic compound its properties when it is used as a solvent are more like those of an organic substance. Very few inorganic substances are soluble in it. A second reason is that its boiling point of -60°C . requires either that the experiments be carried out at a low temperature or that the material be sealed in tubes and the work be done at room temperature with a pressure of about twenty atmospheres.

PREPARATION OF LIQUID HYDROGEN SULFIDE

The apparatus used for the preparation of the liquid hydrogen sulfide is that of Antony and Magri as modified by Quam, and is shown in figure 1. The apparatus consists of three parts: a generator for preparing the hydrogen sulfide, a purifying train, and a liquefaction and delivery reservoir.

The gas is generated in the usual manner by the reaction of dilute hydrochloric acid on ferrous sulfide. The acid is stored in C, the sulfide in G and between the generator and the drier a pressure gauge is placed for protection against excess pressure.

The gas is washed by passing it through water in two Dreschel bottles, K₁ and K₂, which remove any hydrochloric acid spray that may have been carried over. It is next partly dried by passing through two bottles, L₁ and L₂, containing calcium chloride and then through two bottles, N₁ and N₂, containing aluminum sulfide, and finally through two bottles, O₁ and O₂, containing phosphorus pentoxide. The aluminum sulfide not only removes

water left from the calcium chloride, but also in doing so liberates an equivalent amount of hydrogen sulfide and takes out any hydrogen chloride set free from the calcium chloride.

The condenser is in the form of a glass coil which is cooled by immersing it in a mixture of ether and solid carbon dioxide contained in a Dewar tube. By raising the levelling bulb it is possible to get slightly more pressure for the liquefaction. The liquid collects in the larger part of the condenser P and may be forced

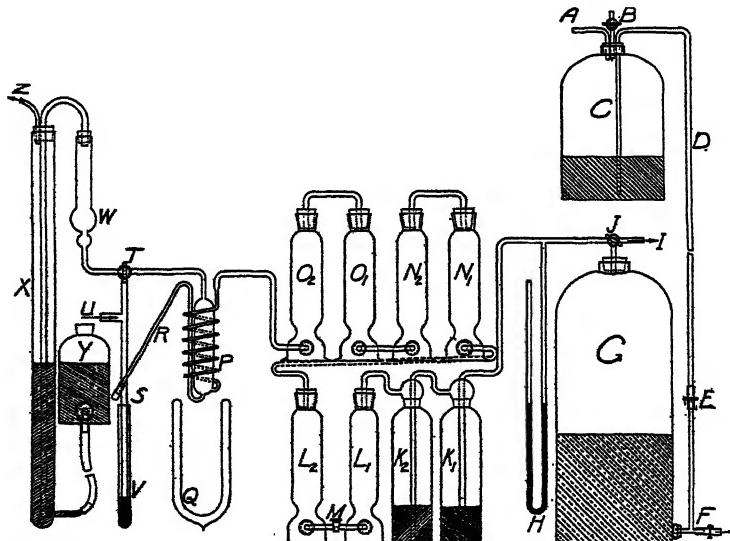


FIG. 1. APPARATUS FOR THE LIQUEFACTION OF HYDROGEN SULFIDE

out from it into storage tubes or reaction tubes by closing the stop-cock T and allowing the gas pressure from the generator to force the liquid out through the tube R. If it does not start readily, it is only necessary to lower the Dewar tube for a little while, thus raising the temperature somewhat, whereupon the vapor pressure of the liquid will force the liquid out.

PHYSICAL PROPERTIES OF LIQUID HYDROGEN SULFIDE

The physical constants of liquid hydrogen sulfide are as follows: melting point, -82.9° ; boiling point, -59.4° ; critical tempera-

ture, 100.4°; critical pressure, 89 atmospheres; density of the liquid at -60°, 0.96; dielectric constant, 10.2; viscosity, 0.00412 dynes per centimeter; surface tension, 25.434 dynes per centimeter.

LIQUID HYDROGEN SULFIDE AS A SOLVENT

The first extensive study of the solvent power of liquid hydrogen sulfide was made by Walker, McIntosh, and Archibald (1), who reported the solubility of some ninety organic compounds including hydrocarbons, halides, acids, esters, acyl chlorides, nitriles, alcohols, ethers, aldehydes, ketones, nitro and amino compounds.

Antony and Magri (2) reported that as a solvent liquid hydrogen sulfide does not resemble water very much but resembles more carbon disulfide, alcohol, ether and benzene. Salts of metals with strong acids are insoluble in it, while nonmetallic substances in general are soluble. The halides of the nonmetals, nitrogen compounds such as naphthylamine and nitronaphthalene, hydrocarbons, alcohols and ethers are soluble. Salts of metals such as copper and lead, and oxidizing agents such as chromates, dichromates, chromic acid and permanganates, are unchanged in liquid hydrogen sulfide. Solid bromine reacts and iodine dissolves to give a dark red solution.

Quam (3) repeated some of this work and extended it to a large number of compounds, both organic and inorganic. In his study he divided the compounds into four groups according to their ability to dissolve in hydrogen sulfide and react with it. His work brought out very well the fact that the substances which dissolve best in liquid hydrogen sulfide are organic in character.

The first group, containing the substances which are soluble without apparent reaction, consists chiefly of organic compounds, with the exception of hydrogen chloride, hydrogen bromide, zinc chloride and a few sulfides. Aluminum chloride should be in this group instead of the last, as it is somewhat soluble and nonreactive.

In the second group are given the compounds that are soluble and react for one of three reasons: (a) because of their oxidizing power; (b) because of the presence of an amino group; or (c) because thiohydrolysis takes place.

The third group contains those elements and compounds that are insoluble but react with the hydrogen sulfide. Any reaction is due either to oxidation or to the formation of a sulfide of the metal.

The last group, which contains the substances which are insoluble and nonreactive, consists almost entirely of inorganic bodies, with the exception of the carbohydrates. Contrary to what one might expect, the sulfides of both metals and nonmetals are insoluble in hydrogen sulfide, with the exception possibly of ammonium hydrosulfide, and its solubility is quite low. Another striking thing shown by the table is the insolubility of the metal salts, such as the chlorides, and the solubility of the chlorides of the nonmetals in groups four, five and six of the periodic table. With the exception of carbon tetrachloride these chlorides are all hydrolyzed in water, some of them almost completely. In liquid hydrogen sulfide, however, they are quite stable except when their oxidizing power causes decomposition. Ralston determined the solubility of thiol and dithio organic acids in liquid hydrogen sulfide and found that they were all very soluble.

CONDUCTANCE IN LIQUID HYDROGEN SULFIDE

The conductance of pure liquid hydrogen sulfide was determined first by Magri (4), who reported the specific conductance as less than 4.1×10^{-7} . Quam and Wilkinson (5), using a very sensitive galvanometer and a 110 volts D.C. current, reported it as less than 1×10^{-11} . Satwalekar, Butler and Wilkinson (6) with a similar set-up obtained a value of 3.7×10^{-11} . The conductance of a good grade of conductivity water is about 0.7×10^{-6} , showing that the value for the conductance of pure water is about 20,000 times greater than it is for liquid hydrogen sulfide.

Walker, McIntosh, and Steele (7) tried the conductance of solutions in hydrogen sulfide of a very large number of organic compounds, and found them all to be very poor conductors. The only solutions that showed appreciable conductance were those of piperidine, pyridine, quinoline and nicotine. These substances are all basic in nature and with the hydrogen sulfide form addition compounds which are sulfides and which are dissociated. Ace-

tonitrile, aldehydes, and ketones unite with hydrogen sulfide and therefore their solutions conduct slightly. Antony and Magri (2) tried the conductance of a number of substances in liquid hydrogen sulfide and found that many nonmetallic compounds are soluble and give conducting solutions: a solution of iodine is a good conductor; one of phosphorus triiodide is less good; solutions of chloroform and of tetramethylammonium chloride also conduct. Chipman and McIntosh (8) found that solutions of iodine and of antimony trichloride showed good conductance, as did also those of tripropylamine and isobutylamine. The curves

TABLE I

Conductances of saturated solutions of some inorganic halides in liquid hydrogen sulfide

SUBSTANCE	($\lambda \times 10^6$)
HgCl ₄	0.031
SnCl ₄	0.106
SiCl ₄	0.129
ZnCl ₄	0.634
HCl.....	0.881
AsCl ₃	1.15
AlCl ₃	2.09
HgBr ₂	5.16
HgI ₂	9.99
FeCl ₃	209.9
SbCl ₃	424.0
0.02 N KCl (in H ₂ O).....	2397.0

show that the equivalent conductance increases with increase in concentration, which is the reverse of what takes place in water solutions. With iodine the usual form of curve is obtained. When equivalent conductance increases with concentration it is assumed that a compound has been formed between the solute and the solvent. Walden (9) accounted for the conductance of the iodine solutions of this nature by the ionization of the iodine molecule into I⁺ and I⁻, which conduct the current.

Because of the very high resistance of solutions in liquid hydrogen sulfide, special cells were constructed with low cell constants. The platinum electrodes were 18 mm. square and were separated and held about 2 mm. apart by small glass rods fused between

them. The unit was held rigidly in place by forcing a small glass tube in between the electrode and the cell wall.

The specific conductances of saturated solutions of a few of the inorganic bodies which are soluble in liquid hydrogen sulfide were determined by Ralston (10) and are given in table 1. For comparison the conductance of a 0.02 *N* KCl solution in water is given.

TABLE 2
Conductances of halides in liquid hydrogen sulfide ($\lambda \times 10^3$)

MOLAR CONCENTRATION	PCl ₃	PBr ₃	AsCl ₃	SbCl ₃
0.01			1.2343	165.7
0.05				583.6
0.0538		0.6643		
0.069	0.2055			
0.1			1.6387	14148.3
0.1086		0.365		
0.162		0.1679		
0.20			1.918	
0.2155				

TABLE 3
Conductances of solutions of alkyl ammonium chlorides ($\lambda \times 10^3$)

MOLAR CONCENTRATION	CH ₃ NH ₂ Cl	(CH ₃) ₂ NH ₂ Cl	(C ₂ H ₅) ₂ NHCl
0.0104			1532.7
0.0113		641.5	
0.0133	21.25		
0.0140			8357.0
0.0340		15256.0	
0.0448			42969.0
0.0468	78.42		
0.0508			66004.0
0.0544	132.6		

The equivalent conductances of the chlorides of the phosphorus family were determined by Quam (5) and the values are shown in table 2. These chlorides are all hydrolyzed readily in water, but in liquid hydrogen sulfide many of them are stable without undergoing thiohydrolysis. The conductance increases with the increase in the atomic weight of the element until bismuth is reached,

but the conductance of bismuth trichloride in liquid hydrogen sulfide is zero. The conductances of both the arsenic and the antimony chlorides increase with increasing concentrations, as was found by Chipman and McIntosh (8).

Since the solubility of ammonium chloride in liquid hydrogen sulfide is practically zero, the conductance is also zero. If the hydrogen atoms are substituted by alkyl groups, the solubility increases and the conductance also. The values obtained by

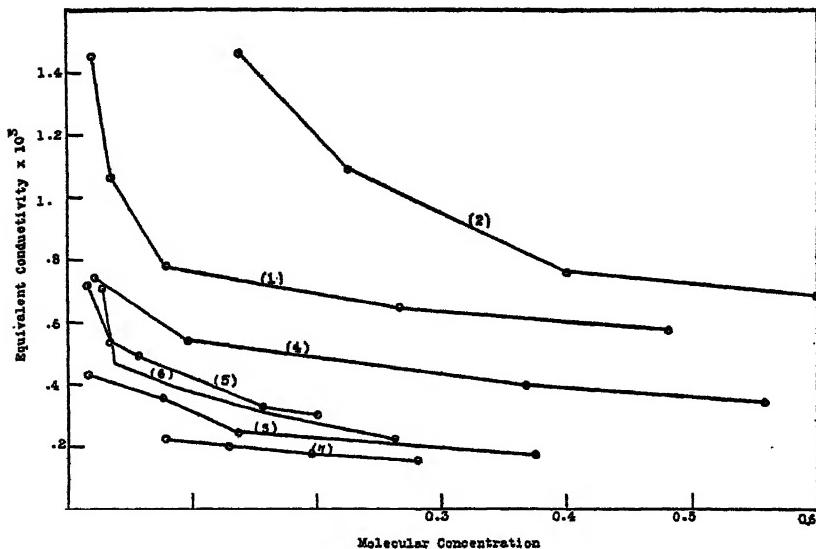


FIG. 2. EQUIVALENT CONDUCTANCES OF THIO ACIDS IN LIQUID HYDROGEN SULFIDE

(1) Thiolacetic acid; (2) dithioacetic acid; (3) dithiopropionic acid; (4) dithiobutyric acid; (5) dithiovaleric acid; (6) dithioisocaproic acid; (7) dithiobenzoic acid.

Quotients for the equivalent conductances (5) of hydrogen sulfide solutions of alkylammonium chlorides are shown in table 3.

CONDUCTANCE OF THIOACIDS

A number of thiol and dithio organic acids were prepared and their equivalent conductances in hydrogen sulfide were determined by Ralston at different dilutions. The results obtained are shown in the curves in figure 2.

In the homologous series the conductance decreases with increasing molecular weight with the exception of propionic acid, whose conductance is apparently lower than it should be for the series. However the same is true for the oxygen acids dissolved in water as the value for propionic acid is low there also.

For comparison, these values and the values for the similar oxygen acids dissolved in water at similar dilutions (32 liters) are given in table 4. It will be observed that the values for the oxygen acids in water solutions are from 1000 to 10,000 times greater than for the thio acids in liquid hydrogen sulfide. Benzoic acid dissolved in water is a fairly strong acid, but the dithiobenzoic

TABLE 4
Comparison of the conductance of thio acids and oxygen acids

IN WATER	λ	IN HYDROGEN SULFIDE	$\lambda \times 10^3$
Acetic.....	8.7	Dithioacetic.....	1.00
Propionic.....	7.4	Dithiopropionic.....	0.41
Isobutyric.....	7.9	Dithiobutyric.....	0.72
Valeric....	7.7	Dithiovaleric.....	0.57
Isocaproic.....	7.5	Dithiocaproic.....	0.62
Benzoic.....	22.3 (64)	Dithiobenzoic.....	0.22
Thiolacetic.....	42.1	Thiolacetic.....	1.29

acid dissolved in hydrogen sulfide is quite weak. Thiolacetic acid is a much stronger acid when dissolved in water than when it is dissolved in hydrogen sulfide.

The decomposition voltage of a solution of antimony chloride dissolved in liquid hydrogen sulfide is $1.9 \pm .1$ volts. None of the other halides soluble in liquid hydrogen sulfide gave a reproducible value for its decomposition voltage.

THIOHYDROLYSIS

Thiohydrolysis of chlorides

The metathetical reaction between hydrogen sulfide and other substances has been called thiohydrolysis to bring out its relationship to hydrolysis. The reaction with the chlorides, especially those of the elements in the fourth, fifth and sixth groups of

the periodic table, brings out the similarities and also the differences in the two reactions. Carbon tetrachloride is insoluble in water but miscible with liquid hydrogen sulfide in all proportions, and reacts with neither of the solvents. All of the other chlorides in the fourth group hydrolyze in water to form the insoluble hydrated oxides, while in hydrogen sulfide they all dissolve with but very little action.

In the phosphorus family the chlorides of phosphorus and arsenic hydrolyze in water to form soluble acids, while the chlorides of antimony and bismuth hydrolyze to form the insoluble

TABLE 5
Thiohydrolysis of halides

COMPOUND ADDED	COMPOUND FORMED BY THIOHYDROLYSIS	COMPOUND FORMED BY HYDROLYSIS
SiCl ₄	SiCl ₄ is soluble in H ₂ S, but does not react	Si(OH) ₄ SiO ₂ + 2H ₂ O
TiCl ₄	2TiCl ₄ ·H ₂ S TiCl ₂ + S	Ti(OH) ₄ TiO ₂ + 2H ₂ O
SnCl ₄	SnCl ₄ dissolves in H ₂ S. There is a slight reaction forming SnS ₂	Sn(OH) ₄ SnO ₂ + 2H ₂ O
PCl ₃	P ₂ S ₃	P(OH) ₃
PCl ₅	PCl ₃	POCl ₃
AsCl ₃	As ₂ S ₃	AsOCl·H ₂ O H ₂ AsO ₃
SbCl ₃	SbSCl·7SbCl ₃	SbOCl
SbCl ₅	SbSCl ₃	H ₂ SbO ₄ SbOCl ₃
BiCl ₃	BiSCl·BiCl ₃	BiOCl
SeCl ₄	SeSCl ₄ Se ₂ Cl ₂ Se + S	H ₂ SeO ₃
TeCl ₄	TeCl ₂ + S Te + S	H ₂ TeO ₃

oxychlorides. In hydrogen sulfide phosphorus trichloride reacts very slowly at low temperatures, but at room temperatures both phosphorus trichloride and arsenic trichloride react to give the sulfides immediately. Antimony trichloride dissolves at room temperature but does not react in the liquid state, although it will react with the vapor when the liquid is removed to form the compound SbSCl·7SbCl₃. Bismuth trichloride reacts immediately with the hydrogen sulfide to form the compound BiSCl·BiCl₃.

The chlorides of elements in the sixth group of the periodic table are all hydrolyzed in water and thiohydrolyzed in hydrogen

sulfide. None of the products formed are simple, but those resulting from the thiohydrolysis are the more complex because of the reducing power of the hydrogen sulfide. A comparison of these reactions is given in table 5.

Thiohydrolysis of esters (11)

Just as esters are hydrolyzed in water solution so are they thiohydrolyzed in liquid hydrogen sulfide. In order to make the comparison more complete the esters of thiolacetic acid were used

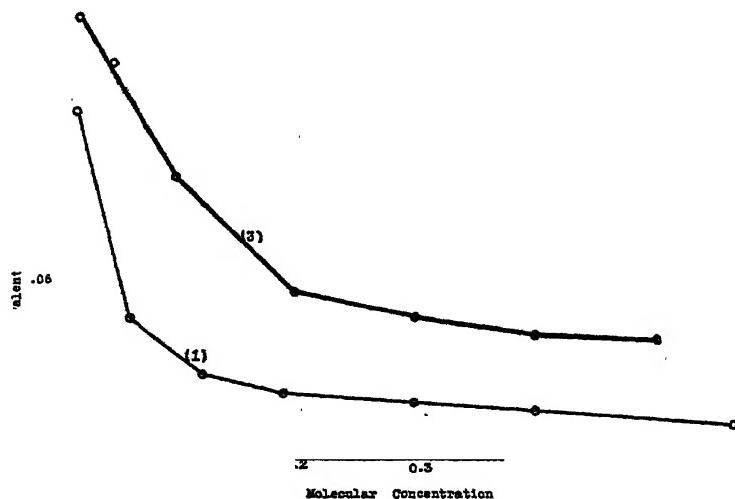


FIG. 3. HYDROLYSIS OF METHYL THIOLACETATE
(1) Ester; (2) ester plus mercaptan; (3) per cent hydrolysis.

rather than the oxygen acid esters. The products of the reactions are the thiol acids and the thioalcohols or mercaptans. The degree of hydrolysis was measured by determining the conductance of the solution of the ester alone in hydrogen sulfide and then of the ester solution saturated with the mercaptan. The calculation of the degree of hydrolysis is made by the following expression

$$\frac{\lambda_0 - \lambda}{\lambda_0 - \lambda_s} = h$$

where h is the degree of hydrolysis, λ_e is the conductance of the ester solution, λ_a the conductance of the acid of the same concentration and λ the conductance of the ester solution saturated with the mercaptan. The mercaptan was found to be a nonconductor when dissolved in the hydrogen sulfide. The value λ is therefore the conductance of the unhydrolyzed ester. λ_e is the conductance of the ester which has not been thiohydrolyzed, increased by the conductance of the acid that has been formed by the thiohydrolysis, while λ_a is the value for the conductance if all of the ester has been thiohydrolyzed. The curves which were obtained for the thiohydrolysis of methyl thiolacetate are given in figure 3, and the data for several of the other esters which were studied are

TABLE 6
Thiohydrolysis of esters of thiolacetic acid in liquid hydrogen sulfide

	PER CENT OF HYDROLYSIS		
	10 liters	5 liters	2 liters
Methyl thiolacetate.....	4.52	2.80	1.22
Ethyl thiolacetate.....	10.10	8.56	5.24
<i>n</i> -Propyl thiolacetate.....	49.3	17.2	11.7
Isopropyl thiolacetate.....	30.8	20.5	18.2
<i>n</i> -Butyl thiolacetate.....	68.0	56.7	53.3

given in table 6. It is to be noticed that as the molecular weight of the ester increases the per cent of thiohydrolysis increases.

REACTIONS WITH LIQUID HYDROGEN SULFIDE

There is some disagreement as to the reaction between liquid sulfur dioxide and liquid hydrogen sulfide. It is well known that in the gaseous condition they react to form water and free sulfur. Lang and Carson (12) reported that when the two substances were mixed and kept cold, sulfur separated out. Mathews (13) reported that unless some third substance, such as water, alcohol, ether, amyl alcohol, acetone, amyl acetate or ethyl acetate is present in the liquid phase to act as a solvent, no reaction takes place. It has been the experience in this laboratory that the two

liquids do not react at low temperatures but that as the temperature rises they react with explosive violence.

The hydrogen sulfide and sulfur dioxide were dried with phosphorus pentoxide and the tubes were cleaned and dried with alcohol and ether and then dried by passing warm air, dried with phosphorus pentoxide, through them. The sulfur dioxide and hydrogen sulfide were condensed directly in these dried tubes which had been cooled to $-78.5^{\circ}\text{C}.$, and the tubes were sealed immediately. As long as the tubes were kept cold no reaction took place, but when the tubes were removed from the cooling bath and the temperature allowed to rise every tube exploded. Such a tube was placed behind a glass screen and the progress of the reaction watched. Within five minutes from the time it was removed from the cooling bath it exploded. The reaction started at the boundary of the two liquids and increased in violence until the pressure was great enough to burst the tube.

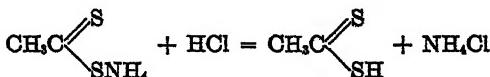
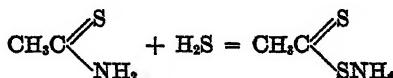
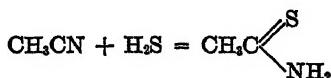
Calcium oxide reacts with liquid hydrogen sulfide to form a mixture of calcium sulfide and calcium hydrosulfide. The mixture loses hydrogen sulfide very readily and one cannot obtain a homogeneous sample. Similarly, calcium carbide reacts with hydrogen sulfide to liberate acetylene and form a white solid which analysis indicates is calcium sulfide. There is also formed a small amount of a light yellow liquid which was thioacetaldehyde.

Cahours (14) treated furfuralamide, dissolved in alcohol, with hydrogen sulfide gas and obtained a yellow crystalline substance whose analysis indicated it to be thiofurfural ($\text{C}_{10}\text{H}_4\text{S}_2\text{O}_2$). Baumann and Fromm (15) isolated a similar substance by passing hydrogen sulfide into an alcoholic solution of furfural. If the two liquids are sealed in a tube and allowed to come to room temperature and then to stand for a few days the mixture solidifies to a yellow crystalline mass. Two samples of this material, recrystallized from ethyl acetate, melted between 95° and 98° and contained 28.74 per cent and 28.57 per cent of sulfur. Baumann and Fromm reported a melting point of $90\text{--}92^{\circ}$. The theoretical per cent of sulfur in thiofurfural is 28.6.

Furan, furfuralcohol, pyromucic acid and the ethyl and nitro-

ethyl esters of pyromucic acid all were treated in a similar manner with hydrogen sulfide. None of them reacted with it, thus indicating that it is the aldehyde oxygen that is replaced and not the oxygen in the ring. Borgeson (16) has shown that this reaction is characteristic of all aldehydes. He has also shown that acyl halides first undergo thiohydrolysis, and that the oxygen is then replaced by sulfur to give either the dithio acid or its anhydride.

Nitriles also thiohydrolyze to give the dithio acids. This is one of the easiest methods of preparing the dithio acids. The reaction is catalyzed by the presence of hydrochloric acid just as is the similar reaction with water. The reaction may be represented as follows:



Since the ammonium chloride is insoluble in liquid hydrogen sulfide it separates out in the tube as a white solid, while the dithio acids are soluble. The following nitriles have been found to undergo this reaction: aceto-, propio-, valero-, succino- and benzo-nitrile; of these the last one reacted the most rapidly and gave the highest yield.

The Grignard reagents will react in liquid hydrogen sulfide just as easily as in water. Ethyl magnesium bromide and naphthyl magnesium bromide were treated with liquid hydrogen sulfide and the resulting hydrocarbons identified. The composition of the solid magnesium compound is quite variable, depending entirely upon the concentration of reagents used and the relative amount of hydrogen sulfide.

REFERENCES

- (1) WALKER, McINTOSH AND ARCHIBALD: J. Chem. Soc. **85**, 1098 (1904).
- (2) ANTONY AND MAGRI: Gazz. chim. ital. **35**, 206 (1905).
- (3) QUAM: J. Am. Chem. Soc. **47**, 103 (1925).
- (4) MAGEI: Atti. accad. Lincei **16**, 516 (1907).
- (5) QUAM AND WILKINSON: J. Am. Chem. Soc. **47**, 989 (1925).
- (6) SATWALEKAR, BUTLER AND WILKINSON: J. Am. Chem. Soc. **52**, 3045 (1930).
- (7) WALKER, McINTOSH AND STEELE: Proc. Roy. Soc. (London) **73**, 450 (1904).
- (8) CHIPMAN AND McINTOSH: Proc. Trans. Nova Scotian Inst. Sci. **16**, (Pt. 4), 188 (1927).
- (9) WALDEN: Z. physik. Chem. **43**, 385 (1903).
- (10) RALSTON AND WILKINSON: J. Am. Chem. Soc. **50**, 258 (1928).
- (11) RALSTON AND WILKINSON: J. Am. Chem. Soc. **50**, 2160 (1928).
- (12) LANG AND CARSON: Proc. Chem. Soc. **21**, 158 (1905).
- (13) MATHEWS: J. Chem. Soc. **129**, 2270 (1926).
- (14) CAHOURS: Ann. **69**, 85 (1849).
- (15) BAUMANN AND FROMM: Ber. **24**, 3591 (1891).
- (16) BORGESON AND WILKINSON: J. Am. Chem. Soc. **51**, 1453 (1929).

NON-AQUEOUS REDUCTION REACTIONS

CHARLES A. KRAUS

Department of Chemistry, Brown University, Providence, Rhode Island

Received February 2, 1931

I. INTRODUCTION

The early investigators in the field of non-aqueous solutions were concerned chiefly with the study of the physical properties of such solutions. At the time—some thirty or more years ago—the theory of electrolytic solutions bulked large in the eyes of physical chemists and, while this theory had met with many notable successes, certain inconsistencies had been discovered that not only halted further progress but also threatened the general acceptance of the theory. It was hoped that the study of solutions—particularly of electrolytes—in non-aqueous solvents might throw light on the problem of electrolytic solutions generally and thus assist in the solution of the problem of aqueous solutions of electrolytes. To what extent these early hopes have been realized I shall not attempt to state, but, in any case, a great many investigators were led to study non-aqueous solutions and an extensive literature of the subject has developed. Perhaps the chief result has been to show that nearly all liquid media are capable of dissolving suitable electrolytes and of forming electrolytic solutions. On the other hand, it is now evident that few solvents are capable of dissolving ordinary salts quite generally.

The problem of electrolytic solution served to focus the attention of chemists on the physical rather than on the chemical properties of non-aqueous solutions. As a result we have, even today, only a fragmentary knowledge of the chemistry of solutions in solvents other than water. But, however complete our knowledge of the physical properties of non-aqueous solutions may be, these solutions will play only an unimportant rôle in

chemistry unless it can be shown that such solutions enable us to carry out important chemical reactions and processes that are not possible otherwise. As yet, little has been done to develop systematically the chemistry of solutions in non-aqueous solvents. Until this has been done it will be difficult to predict what will be the future of the chemistry of non-aqueous solutions. It is evident, however, that the number of solvents that give promise of extensive development as media for chemical reactions is very limited. Such solvents must be available in quantity at low cost, must permit of fairly ready manipulation and, finally, must be capable of dissolving a variety of chemically active substances. At the moment, only liquid ammonia and liquid sulfur dioxide seem to meet these requirements.

Despite this limited choice of non-aqueous solvents, there is good reason for believing that many reactions can be carried out more conveniently and efficiently in certain non-aqueous solvents than in water. To illustrate the importance of studying the chemistry of non-aqueous solutions, I shall discuss certain reduction reactions in liquid ammonia. Similar reactions may be carried out in other solvents, particularly the primary aliphatic amines. Although our knowledge of these reactions is still quite incomplete, the material already available is too extensive to permit of a full discussion here. It is hoped, however, to show that many new reactions are made possible by the use of liquid ammonia as a solvent and that new points of view are presented with respect to otherwise familiar reactions.

Liquid ammonia and the lower primary amines are unique in their power to dissolve the alkali metals and the metals of the alkaline earths (1). It has been shown that in these solutions the metals are ionized into normal positive ions and negative ions which are the same for all metals (2). These negative ions have been identified as electrons in solution. As was to have been expected, the electrons in solution are very active chemically, and, since the combination of an electron with an atom constitutes reduction, these metal solutions constitute the strongest reducing agents that we have at our disposal for reduction in a homogeneous phase.

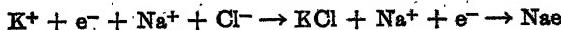
It may be pointed out that, while water is well adapted as a medium in which to carry out reactions with strong oxidizing agents, it is not well adapted to carrying out reactions with strong reducing agents. The reducing power of reducing agents in water is limited by the fact that stronger reducing agents than hydrogen set hydrogen free from water.

The strongest reducing agents that we have are the alkali metals and, since these metals either react with nonmetallic solvents or are insoluble in them, reductions by means of the alkali metals are greatly restricted in their application. As a result, the field of reduction reactions by means of strong reducing agents remains largely unexplored. In the study of such reactions, the solutions of the metals in liquid ammonia offer the only available means for systematic investigation.

The process of reduction by means of metals in liquid ammonia consists essentially in the combination of an electron with an atom (or group of atoms) or an ion. The positive ions of the metals are, of course, also present but they take no part in the reaction. The positive ions possess no metallic properties, and they are concerned in the process of reduction only to the extent of supplying the counterbalancing electrical charge for negative ions. When an atom undergoes reduction, a negative ion is formed. When a positive ion is reduced a neutral atom or group is commonly formed. Below are discussed some typical examples of various types of reduction reactions.

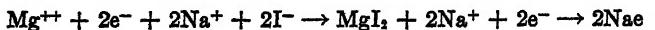
II. METATHETIC REACTIONS

There are numerous reactions of the metals that are of the metathetic type. If we add an alkali metal, M_1e , to a salt, M_2X , of another metal, we have in solution the ionic species: M_1^+ , M_2^+ , X^- and e^- , where e^- represents the electron. By metathesis, four binary ionic combinations are possible (3), namely: M_1X_1 , M_2X_2 , M_1e and M_2e . No reaction will occur unless one of the binary products is removed from solution. For example, on treating sodium chloride with metallic potassium, we have the possible reaction:



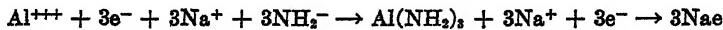
If the solution is dilute, no reaction occurs; but, if the solution is concentrated, reaction takes place from left to right because potassium chloride is the least soluble of the four possible binary products and is precipitated. The sodium ions, together with the electrons, remain in solution, from which they may be recovered as metallic sodium by evaporating the solvent.

A better example of metathesis is the reduction of sodium from sodium iodide by means of magnesium (4). Here the reaction proceeds as follows:



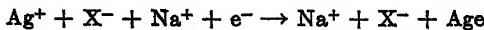
Sodium is reduced from its iodide by means of magnesium because of the very low solubility of magnesium iodide, even though metallic magnesium is only slightly soluble in liquid ammonia.

A corresponding reduction occurs when sodium (or potassium) amide is acted upon by less electropositive metals that are not measurably soluble in liquid ammonia, such as aluminum (5). Thus we have the reaction:



The reaction proceeds as it does because of the very low solubility of aluminum amide. Similar reactions occur in the case of other elements such as beryllium, manganese and zinc.

Another type of metathetic reaction is that in which a weakly positive metal is reduced from its salt by means of an alkali metal (3). The reduction of silver from its salts by means of sodium may serve as example (6). Here we have the ionic reaction:

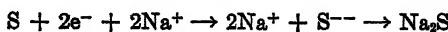


The symbol Age represents neutral or metallic silver. The reaction occurs because of the low solubility of metallic silver, Age.

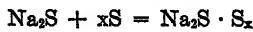
III. REACTION OF ELECTRONS WITH NEGATIVE ELEMENTS

If an electronegative element is introduced into a solution containing electrons (metal), the atoms of this element combine

with the electrons to form negative ions. In other words, a salt is formed. Thus, for example, in the case of sulfur (7), we have the reaction:



Sodium sulfide is only slightly soluble in liquid ammonia and is therefore precipitated. It should be pointed out, however, that the normal sulfide combines readily with additional sulfur atoms to form soluble polysulfides, thus:



Sodium sulfide and the polysulfides are well known and the reaction of electrons with sulfur presents nothing essentially new. However, while in aqueous solution the representatives of the poly type of salts are very limited, in liquid ammonia they are quite numerous. They may all be formed by the direct interaction of electrons with electronegative or amphoteric elements (8). For example, if metallic lead is treated with a solution of sodium in liquid ammonia, the electrons are taken up directly by the lead atoms to form a normal plumbide according to the equation:



The normal plumbide, like the corresponding sulfide, is insoluble in liquid ammonia and, like the sulfide, reacts with additional lead atoms to form soluble polyplumbides (9). If lead is present in excess, we have the reaction:



The compound, Na_4Pb_9 , is a metallic alloy in the pure state. It may be obtained from the solution by evaporating the solvent, and it is redissolved when the solvent is again added. Although this polyplumbide is a metallic alloy in the pure state, it is a true salt in solution. If a solution of the plumbide, Na_4Pb_9 , is electrolyzed between lead electrodes, $2\frac{1}{2}$ atoms of lead are dissolved at the cathode and precipitated on the anode per equiva-

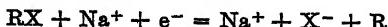
lent of electricity (10). This shows conclusively that the plumbide in solution is a true electrolyte and that it comprises an anion in which nine atoms of lead are associated with four negative charges of electricity.

Just as the metallic elements, when dissolved in liquid ammonia, dissociate into normal positive ions and a common negative ion, the electron, so the metallic compounds, when dissolved in ammonia, dissociate into normal positive and negative ions, the latter comprising the more electronegative constituent of the compound.

Soluble salts of the poly type are formed quite generally by the direct interaction of electrons with the elements of the fourth, fifth and sixth groups (11). The only exceptions are silicon, carbon and perhaps oxygen. Oxygen reacts directly with the alkali metals in liquid ammonia solution to form, in the first instance, dioxides which, in the case of potassium, rubidium and cesium, are further oxidized to tetroxides (12).

IV. REACTION OF ELECTRONS WITH NEGATIVE ELEMENTS IN ORGANIC COMPOUNDS

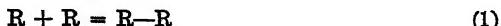
If we have a compound containing electronegative elements not in the ionic condition, reaction ordinarily takes place with electrons, the negative elements going into the negative ionic condition (13). Familiar examples are the organic halides of the type RX, where R is an organic group and X is a halogen. Such compounds react with the alkali metals in liquid ammonia solution according to the equation:



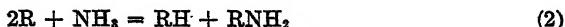
The halogen invariably goes into the state of a negative ion and remains in solution, or is precipitated in the form of the corresponding salt of the alkali metal. This reaction of electrons with halogens occurs even in the case of highly halogenated organic compounds and serves as a convenient method for the analysis of organic compounds for the halogens (14).

The organic residue, R, reacts variously, depending upon its

nature. In some instances, two groups polymerize (15) according to the equation:



More frequently, however, the organic group reacts with the solvent. The final products are in accordance with the reaction equation:



It is possible that in some instances the mechanism of the reaction actually corresponds to that of equation 2. It is probable, however, that in most cases, reaction takes place as follows:



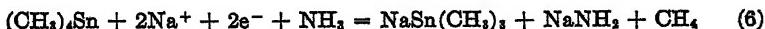
In other words, a salt of the organic group is formed (15, 16). Such salts are not usually stable in the presence of liquid ammonia and ammonolysis occurs according to the equation:



The resulting amide in turn reacts with the original organic halide according to the equation:

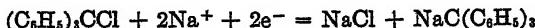


We have some direct evidence that the organic group, R, does not react directly with ammonia according to equation 2. For example, when tetramethyl tin is treated with sodium in liquid ammonia, we have the reaction:

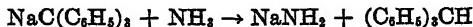


Since, in this reaction, there is present no substance with which sodium amide reacts, the formation of the amide becomes evident (17). It is probable that in other reactions, such as those of the organic halides, the hydrocarbon is formed, as it is in the case of tetramethyl tin, by ammonolysis according to equation 4, while an amine is formed as the result of the action of sodium amide on the original unreacted halide according to equation 5.

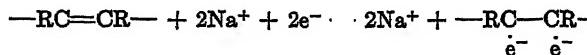
In the case of certain aromatic carbon derivatives, the intermediate formation of a more or less stable sodium salt is clearly evidenced by the formation of colored solutions, the color being due to the presence of these salts (18). This is, for example, the case with triphenylmethyl derivatives. If triphenylmethyl chloride is reduced by means of sodium, the following reaction occurs:



Sodium triphenylmethide is soluble in liquid ammonia, to which it imparts an intense red color. If this solution is evaporated, there are left behind, in addition to sodium chloride, sodium amide and triphenyl methane (19). In other words, ammonolysis occurs with the generation of the hydrocarbon and sodium amide according to the equation:



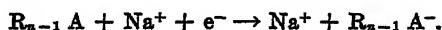
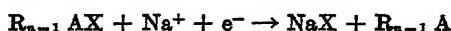
With the exception of triarylmethanes, the electrons do not react with the saturated hydrocarbons (20). They do not react with the doubly bonded aliphatic hydrocarbons, but they frequently react with aromatic hydrocarbons containing double bonds. Thus, they react with diphenyl (21) and with naphthalene (22). What happens is that the double bond is broken according to the equation:



There is formed a disodium salt which, in general, will be more or less completely ammonolyzed. Frequently one of the valences undergoes ammonolysis, while the other remains stable. Mention might also be made of the fact that the aryl ketones react with the alkali metals to form solutions of the metal ketyls (23). The alkali metals react with many organic compounds containing sulfur, selenium, nitrogen, phosphorus, tin, lead, etc. The organic oxygen compounds are, in general, rather stable toward the electrons in ammonia. Thus, the alkali metals do not react with the ethers and, in many cases, probably not with the carboxyl group of acids (20).

V. REACTION OF ELECTRONS WITH COMPOUNDS CONTAINING AMPHOTERIC ELEMENTS

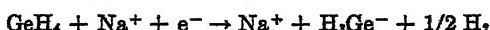
An amphoteric element A of valence n , of which $n-1$ valences are satisfied by hydrocarbon groups, forms compounds of the type $R_{n-1}AX$, where X is an electronegative element or group. Such compounds are reduced to the free state by means of electrons in liquid ammonia. The free radicals, $R_{n-1}A$, may be further reduced to salts of the type $NaAR_{n-1}$. The reactions may be written as follows:



Salts of the type $NaAR_{n-1}$ are well known in the case of oxygen. The alcoholates and phenolates are familiar examples. Salts of oxygen acids belong to this class of substances, the organic substituent consisting of an $RCO-$ group. Corresponding compounds of other elements are not well known. The mercaptides, $RSNa$, are known, but the corresponding compounds of elements of the fourth and fifth groups have received little attention. It may be stated, however, that all the elements of the fourth, fifth and sixth groups form stable salts of the type $NaAR_{n-1}$ and many of them form the free groups, AR_{n-1} , which usually exist in the bimolecular form.

In recent years groups of the type R_nA , derived from the elements of the fourth group, have been extensively studied. The groups in the free state resemble the triarylmethyls. They form salts with the alkali metals in liquid ammonia that are analogous to the metal alkyls of Schlenck. For the elements silicon (24), germanium (25), and tin (26), organic derivatives corresponding to the metal alkyls have been prepared by reduction by means of the alkali metals in liquid ammonia or ethylamine.

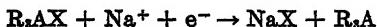
In the case of germanium, sodium germanyl, $NaGeH_3$, the analog of sodium methyl, has been prepared (27). Monogermane, GeH_4 , reacts directly with sodium in liquid ammonia to form sodium germanyl according to the equation:



Sodium germanyl is a colorless salt which is readily soluble in liquid ammonia and whose solutions conduct the electric current with considerable facility. It is entirely stable in solution and shows no tendency to ammonolyze. The corresponding derivatives of heavier elements of the fourth group would probably not prove stable.

Since compounds of the type R_3ANa have been prepared in the case of silicon, germanium and tin, it is of interest to compare their properties. The silicon derivatives show a markedly greater tendency to ammonolyze than do the corresponding derivatives of germanium or tin. Sodium germanyl is much more stable toward ammonolysis than is sodium triethylgermanide (28).

If a compound of the type R_3AX is completely reduced by means of sodium, reduction takes place in two stages. First, the neutral group is formed according to the reaction equation:



Then the group AR_3 is further reduced to the state of a negative ion according to the equation:

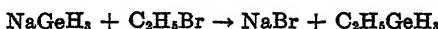


These compounds are true salts in which the groups AR_3^- act as anions. They are very highly ionized in liquid ammonia. Thus, the conductance of sodium triphenylmethide, sodium triphenylgermanide and sodium triphenylstannide has been measured in liquid ammonia solution (29). The results indicate an ionization greater than that of ordinary salts such as sodium bromide.

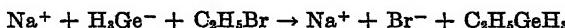
VI. REDUCTION BY MEANS OF WEAKLY ELECTRONEGATIVE IONS

In anions of the type R_3A^- , the electron is not firmly held. The energy effect (heat) accompanying the formation of such anions has been measured in the case of tin and found to amount to only a few thousand calories (30). The salts of such anions therefore serve as convenient reducing agents. In general, they react readily with negative elements or with compounds of such

elements. Thus sodium germanyl reacts with ethyl bromide according to the equation (31):



Here we have a coupling of the germanyl group with the ethyl group. The reaction consists in the interchange of an electron and a C_2H_5 group and may be written thus:

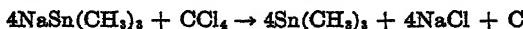


Such reactions are quite common, particularly in the case of the simpler halogen derivatives of hydrocarbons (26). In the case of the more highly halogenated hydrocarbons, the reactions are often more complex, and ammonia may take part in the reaction. Thus, when chloroform is treated with sodium triphenylgermanide, the following reaction occurs:



The reaction is actually the resultant of several coupled reactions that need not be discussed here (33).

When sodium trimethylstannide is treated with carbon tetrachloride in liquid ammonia, reaction occurs as follows:



Trimethyl tin and sodium chloride are formed quantitatively (32). It is not known what becomes of the carbon, but it is safe to assume that it reacts with ammonia. A similar reaction occurs on treating sodium triphenylgermanide with carbon tetrachloride in liquid ammonia (33).

The presence of ammonia may thus complicate the result by entering into reaction with the organic residue. This difficulty may be overcome by dissolving the salt in a neutral solvent, such as benzene or ether. Carbon tetrachloride reacts with sodium triphenylgermanide in benzene solution with the formation of sodium chloride and triphenyl germanium (34). What becomes of the carbon atom of carbon tetrachloride has not, as yet, been determined. Reactions of this type would seem to afford a

means for studying the chemical properties of the free valences of carbon.

Sodium triphenylgermanide reacts with silicon tetrachloride in benzene solution, sodium chloride and triphenyl germanium being formed (35). The silicon atom seems to react with the solvent; but the nature of the reaction remains to be determined. Without doubt salts of the type NaAR_2 will be found to react quite generally with the halogen derivatives of the elements of the fourth, fifth and sixth groups. As yet, this field has not been systematically investigated.

Compounds of the type NaAR_2 , of the elements of the fifth group, are less well known than the corresponding compounds of elements of the fourth group. The nitrogen derivatives are practically the only ones that have been studied. Sodium anilide $\text{NaN}(\text{H})\text{C}_6\text{H}_5$, and sodium diphenylamide, $\text{NaN}(\text{C}_6\text{H}_5)_2$, are excellent salts in liquid ammonia solution, particularly the latter (36). That these and similar compounds of other elements would react with compounds containing strongly electronegative elements or groups seems certain. Here, again, we have a fertile field for further investigation.

VII. CONCLUSION

The importance of non-aqueous solutions in modern chemistry depends upon their availability for the purpose of carrying out reactions that cannot be carried out in aqueous solutions or by other means. With respect to reduction reactions, the solutions of the metals in liquid ammonia afford a unique reagent. The electrons in these solutions are highly reactive toward electronegative and amphoteric elements, and an extensive field of chemistry, not hitherto cultivated, becomes accessible to investigation. The results already obtained serve to broaden our views regarding certain compounds; the elementary metals themselves appear as salts of the electron and certain metallic compounds appear as normal salts of the more electronegative constituent.

Such compounds as the alkali metal alkyls appear as examples of a numerous class of compounds of the elements of the fourth, fifth and sixth groups. They owe their existence to the amphi-

teric nature of these elements, particularly their tendency to go into the state of negative ions. These compounds are highly ionized in solution and constitute valuable reagents for the reduction of other elements or groups.

The systematic investigation of the chemistry of non-aqueous solutions cannot but lead to many valuable results. Substantial progress, however, will be made only when the chemistry of non-aqueous solutions is made a primary objective rather than a side line, incident to other investigations.

REFERENCES

- (1) KRAUS: J. Am. Chem. Soc. **29**, 1557 (1907).
- (2) KRAUS: J. Am. Chem. Soc. **30**, 1323 (1908); **36**, 864 (1914); **43**, 749 (1921); **44**, 1216 (1922).
- (3) KRAUS AND KURTZ: J. Am. Chem. Soc. **47**, 43 (1925).
- (4) BERGSTROM: J. Am. Chem. Soc. **48**, 2848 (1926).
- (5) BERGSTROM: J. Am. Chem. Soc. **45**, 2788 (1923); **47**, 1836 (1925); **50**, 652 (1928).
- (6) BURGESS AND SMOKE: J. Am. Chem. Soc. **52**, 3573 (1930).
- (7) BERGSTROM: J. Am. Chem. Soc. **48**, 146 (1926).
- (8) KRAUS: Trans. Am. Electrochem. Soc. **45**, 175 (1924).
- (9) JOANNIS: Compt. rend. **113**, 797 (1891); **114**, 595 (1892).
KRAUS: J. Am. Chem. Soc. **29**, 1557 (1907).
SMYTH: J. Am. Chem. Soc. **39**, 1899 (1917).
- (10) SMYTH: J. Am. Chem. Soc. **39**, 1899 (1917).
- (11) PECK: J. Am. Chem. Soc. **40**, 335 (1918).
BERGSTROM: J. Am. Chem. Soc. **47**, 1503 (1925).
- (12) RENGADE: Ann. chim. phys. [8] **11**, 348 (1907).
KRAUS AND WHITFORD: J. Am. Chem. Soc. **48**, 1781 (1926).
- (13) KRAUS AND WHITE: J. Am. Chem. Soc. **45**, 768 (1923) for older references.
WHITE AND KNIGHT: J. Am. Chem. Soc. **45**, 1780 (1923).
WHITE, MORRISON AND ANDERSON: ibid. **46**, 961 (1924).
- (14) DAINS, VAUGHAN AND JANNEY: J. Am. Chem. Soc. **40**, 936 (1918).
CLIFFORD: ibid. **41**, 1051 (1919).
- (15) KRAUS AND WHITE: J. Am. Chem. Soc. **45**, 775 (1923).
- (16) WHITE: J. Am. Chem. Soc. **45**, 779 (1923).
- (17) KRAUS AND SESSION: J. Am. Chem. Soc. **47**, 2361 (1925).
- (18) WOOSTER AND MITCHELL: J. Am. Chem. Soc. **52**, 688, 1042 (1930).
- (19) KRAUS AND KAWAMURA: J. Am. Chem. Soc. **45**, 2756 (1923).
- (20) KRAUS AND WHITE: J. Am. Chem. Soc. **45**, 768-78 (1923).
- (21) GREENSTEIN, JESSE H.: Unreported observations made in the Chemical Laboratory of Brown University.
- (22) WOOSTER AND SMITH: J. Am. Chem. Soc. **53**, 179 (1931).
- (23) WOOSTER: J. Am. Chem. Soc. **50**, 1388 (1928).

- (24) EATOUGH, HARRY: Phenyl Silicon Derivatives, Thesis, Brown University, 1929.
- NELSON, WALTER K.: Ethyl Silicon Derivatives. Thesis, Brown University, 1930.
- (25) KRAUS AND FOSTER: J. Am. Chem. Soc. 49, 457 (1927).
KRAUS AND BROWN: ibid., 52, 4031 (1930).
- (26) KRAUS AND GREER: J. Am. Chem. Soc. 44, 2829 (1922); 47, 2588 (1925).
CHAMBERS AND SCHERER: ibid. 48, 1054 (1926).
KRAUS AND BULLARD: ibid. 48, 2131 (1926).
KRAUS AND NEAL: ibid. 52, 4426 (1930).
- (27) CARNEY, E. SEATON: Thesis, Brown University, 1928.
- (28) FLOOD, E. ALLISON: Thesis, Brown University, 1930.
- (29) KAHLER, W. HARLOW: Thesis, Brown University, 1930.
- (30) SCHMIDT, FREDERIC C.: Unreported observations made at Brown University.
- (31) TEAL, GORDON K.: Unpublished observations made at Brown University.
- (32) KRAUS AND NEAL: J. Am. Chem. Soc. 52, 4432 (1930).
- (33) NUTTING, HOWARD S.: Thesis, Brown University, 1928.
- (34) FOSTER, LAURENCE S.: Thesis, Brown University, 1926.
- (35) GLARUM, SIVERT N.: Unpublished observations made at Brown University.
- (36) HAWES, W. W.: Thesis, Brown University, 1930.

A STUDY OF THE REDUCING ACTION OF SODIUM UPON SALTS IN LIQUID AMMONIA SOLUTION

WAYLAND M. BURGESS AND EDWARD H. SMOKER

Department of Chemistry, University of Cincinnati, Ohio

Received January 31, 1931

INTRODUCTION

One of the outstanding properties of a solution of an alkali or alkaline earth metal in liquid ammonia is its powerful reducing action. This property is attributed to solvated electrons, the presence of which as anions has been demonstrated through a study of the electrical properties of metal solutions (1). In accord with this view, the reduction of many substances, when added to these solutions, takes place rapidly and effectively, so that this method offers unusual opportunities for the study of the reduction of all classes of compounds.

This paper describes specific examples of the reduction of inorganic salts by metals in liquid ammonia, with the formation of an insoluble product, usually very reactive towards air and water. Three types of reactions are illustrated: (I) The reduction product is the free metal; (II) The reduction product is an intermetallic compound; (III) The reduction product is the free metal, which takes part in additional reactions involving the solvent.

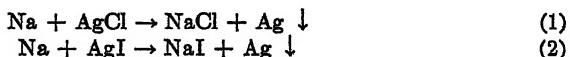
I. THE REDUCTION PRODUCT IS THE FREE METAL

The reduction of silver salts by sodium in liquid ammonia best illustrates the type of reaction in which the salt is reduced to the free metal. Sodium reacts very readily and smoothly with silver chloride, or with silver iodide, forming a black precipitate of metallic silver. No further reaction or evolution of gas takes place (2).

These reactions, and also those described later, were carried out at the temperature of boiling liquid ammonia in an apparatus designed to permit the following: an accurate determination of the amounts of reacting substances; collection and analysis of gases liberated in the course of the reaction; purification, by repeated washings with ammonia, of any precipitate formed; analysis of the precipitate, avoiding reaction with air (3).

The results with silver salts have been checked in two ways: by the ratio of the reacting substances and by analysis of the precipitate. The ratio of gram-atoms of sodium to moles of silver chloride, or of silver iodide, taking part in the reaction can be determined very accurately, the blue color of the dissolved sodium serving as a splendid endpoint. Values of 1.001 using silver chloride and 1.006 using silver iodide were obtained. Analyses of the precipitate gave 98.7 and 98.0 per cent silver for the two cases. The composition of the precipitate is low because of the inability to remove the adsorbed salts completely by washing.

These reactions are represented by the equations:



Other examples of this type of reduction reaction are reduction of cuprous iodide with sodium to free copper (4) and reduction of silver chloride and of silver iodide with potassium to free silver (5).

II. THE REDUCTION PRODUCT IS AN INTERMETALLIC COMPOUND

The reduction of inorganic salts with liquid ammonia solutions of a metal results, in the majority of cases, in the formation of an intermetallic compound of the reduced and reducing metal. The reaction between sodium and zinc cyanide has been studied in considerable detail (3), and the results are here presented as an illustration of this type.

Sodium reacts vigorously with zinc cyanide to form a black metallic precipitate. No gas is evolved. This reaction has been investigated by the determination of the ratio of the reacting materials and by analysis of the precipitate. The ratio

of gram-atoms of sodium to moles of zinc cyanide was found to be 2.22. The average value for the zinc content of the precipitate was 91.8 per cent. Even when the reaction was carried out with an excess of sodium present, the composition of the precipitate did not change. Both the reaction ratio and the analysis indicate the formation of the compound, NaZn_4 , whose theoretical zinc content is 91.9 per cent. Upon treatment with dilute acid, 9.05 equivalents of hydrogen per mole of sodium zinc were obtained, which agrees well with the theoretical yield of 9.0 equivalents for NaZn_4 .

These results establish the following equation for the reaction:



No evidence of the existence of a compound of this nature is found in the study of the freezing point diagram for zinc and tin (6).

As other examples of this type of reaction, the following insoluble compounds were obtained as products of reduction in liquid ammonia by Kraus and Kurtz (7): NaHg , NaHg_2 , NaCd , Na_5Sb , Na_3Bi , Na_3Te_2 , CaAg , Ca_7Zn , Ca_3Hg_2 , Ca_2Pb_3 , and Ca_5Sn . The ratio of reacting substances was used to determine the formula in each case. Identification of these compounds by analysis has not yet been made. In most cases, these compounds have not been found by the method of thermal analysis.

III. THE REDUCTION PRODUCT TAKES PART IN ADDITIONAL REACTIONS

As an illustration of the case where the reduced metal takes part in reactions involving the solvent, the reduction of manganeseous iodide with sodium will be described. Since this work has not been published elsewhere, and no similar reaction has been noted, it will be discussed more in detail than the other types.

The reaction between sodium and manganeseous iodide occurs very readily with the formation of a shiny black precipitate and considerable evolution of hydrogen. Contrary to previous reactions, no quantitative relations between the reacting sub-

stances can be established, all of the sodium being used up irrespective of the amount of manganous iodide employed. Neither is the reaction complete after the free sodium has disappeared, as indicated by the steady production of gas over a period of several days. Two series of reactions occur: the first of these takes place rapidly, with the reduction of the manganous iodide to free manganese, and the free metal thus formed catalyzes the reaction between sodium and ammonia, liberating hydrogen; in the second or slower reaction, the reduced manganese reacts with the sodium amide, formed in the previous steps, and the solvent ammonia.

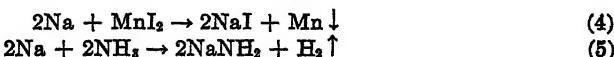
The experimental data for this work will be summarized separately for each series of reactions. There are three facts of importance in establishing the first or rapid reaction: no definite ratio between the reacting substances was found to exist; considerable amounts of hydrogen were liberated; analysis of the precipitate showed that it contained manganese, sodium, and ammonia, as amide, in widely varying proportions.

The reaction ratio, or ratio of the gram-atoms of sodium to moles of the salt, is usually of prime importance in investigating the nature of these reactions. It can normally be determined very accurately. But with manganous iodide the values varied over a wide range; in fact, any amount of sodium could be used for a given amount of manganous iodide. The simple reduction of manganous iodide requires two gram-atoms of sodium to one mole of manganous iodide. But even with a reaction ratio of 30, all of the sodium reacted and the blue color disappeared within a period of half an hour. This establishes the fact that the sodium enters into reactions other than simple reduction of the manganese salt.

The liberation of hydrogen shows that these reactions involve the solvent ammonia. The amount of hydrogen liberated was found to vary directly as the amount of sodium not required for the simple reduction; that is, only a little hydrogen was liberated for a reaction ratio of 2. Here nearly all the sodium entered into the reduction of manganous iodide. But, as the amount of sodium in excess of that required for the reduction increased, the amount of hydrogen increased in the same proportion.

Upon analysis, the precipitate was found to contain varying amounts of manganese, sodium, and amide. These amounts, however, varied in a regular manner, the per cent of manganese reaching a maximum of 90 per cent for reaction ratios of approximately 2. Then, as the ratio increased over 2, the per cent of manganese decreased, and the amount of sodium and amide increased.

These results establish the following reactions as taking place concurrently:

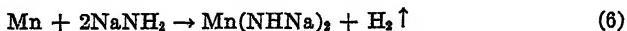


Equation 4 represents the reduction of a metal halide to the free metal, as described under the reactions with silver salts. Equation 5 represents the reaction between sodium and the solvent, ammonia, and accounts for the liberation of hydrogen. For equivalent amounts of sodium and manganous iodide, the reaction proceeds largely according to equation 4. But even with equivalent amounts, the liberation of a small volume of hydrogen and the presence of sodium amide in the precipitate show that reaction 5 occurs concurrently. When the sodium is present in large amounts, all of the excess sodium reacts according to equation 5.

It is remarkable that the excess sodium should react so rapidly with the ammonia. Normally this reaction requires a catalyst and even then is slow. An especially favorable catalyst must be present in this case. It is, undoubtedly, the freshly reduced manganese formed in the initial step of the reaction.

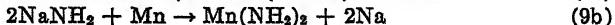
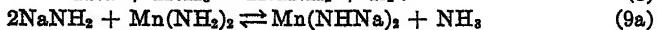
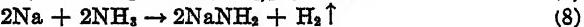
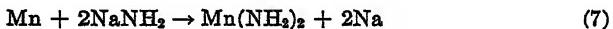
That reactions other than those represented by equations 4 and 5 occur, is established by the slow but steady evolution of hydrogen for a considerable time after the blue color of the sodium has disappeared. Another product of these slow reactions is indicated by the fact that the solution becomes deep yellow. Bergstrom (8) found that potassium ammonomanganite, $\text{Mn}(\text{NH}_2)_2$, dissolves somewhat in liquid ammonia to give a bright yellow solution. The similar color in this reaction, together with the liberation of hydrogen, indicates a reaction

between manganese and sodium amide to form sodium ammonomanganite, as represented by the equation



However, it is not possible to explain the reactions that occur by this equation alone, because the amount of hydrogen liberated is greater than can be accounted for by equation 6. In the reactions that were followed to completion, the initial amounts of sodium and manganous iodide were such that the manganese formed by reduction was considerably in excess of the sodium amide that could be present. Yet the hydrogen collected was found to be closely equivalent to the manganese and in no way dependent upon the amount of sodium amide. This requires the slow reaction to involve not only manganese and sodium amide, but also the solvent ammonia.

It is not possible to establish definitely the steps of the slow reaction on the basis of the work already done. The following series of equations are proposed simply as one possible explanation:



Equations 7, 8, and 9a represent the process of the reaction in liquid ammonia between a metal and a strong base, as developed by Bergstrom (9). These same equations have been established by him using potassium amide instead of the sodium compound (8).

It is assumed that the sodium amide formed in equation 8 reacts in two ways. One reaction, equation 9a, is with manganous amide to form sodium ammonomanganite, the existence of which is shown by the yellow color of the solution. Concurrently, a reaction takes place with manganese, equation 9b, forming again manganous amide and sodium, which is, of course, a repetition of equation 7. This would be followed by a reaction of the sodium with ammonia, liberating hydrogen and regenerating sodium amide. In this way all of the hydrogen obtained

is the result of the reaction of sodium and ammonia. Since the sodium amide is reformed, the amount of hydrogen would depend only on the manganese available, and this is found to be the case.

PROPERTIES OF THE REDUCTION PRODUCTS

In nearly all of the reactions studied, the reduction products have one characteristic property in common: they are particularly reactive towards air and water. Only in the case of silver salts has the product, silver, been non-reactive. Reduced copper prepared in this way becomes incandescent on exposure to air. Manganese sparks vigorously and becomes white hot. The product of the reduction of nickel salts behaves similarly (10). Sodium zinc reacts with explosive violence with either air or water. The compounds prepared by Kraus and Kurtz (7) were all found to be reactive.

Sodium zinc is the only one of these products for which any study has been made of the reaction that occurs with air or water (3). Here it was found that with dry oxygen at low concentrations, the oxidation reaction is divided into two distinct stages. At first, 0.5 gram-atom of oxygen per mole of sodium zinc is rapidly taken up with considerable evolution of heat. This corresponds to the formation of sodium monoxide. A secondary reaction, evidently the oxidation of the zinc, then proceeds at a very slow rate for a long period of time. Increased concentration of oxygen, or air, the presence of moisture, or heating all greatly accelerate the secondary oxidation. With water, a reaction liberating hydrogen continues with decreasing rate for a long time. In one month, approximately four equivalents of hydrogen were collected per mole of sodium zinc.

The excellent ability of the manganese to catalyze the reaction between sodium and ammonia further illustrates the active condition of these reduced metals. This reactivity is probably due to the finely divided state of the substance. On this basis, metals, or intermetallic compounds, formed by reduction in liquid ammonia, are obtained as very minute particles. This method of reduction may well be utilized in the preparation of

metal catalysts, where an activated state or very large surface is essential.

REFERENCES

- (1) KRAUS: J. Am. Chem. Soc. 30, 1323 (1908); 36, 864 (1914).
- (2) BURGESS AND SMOKER: J. Am. Chem. Soc. 52, 3573 (1930).
- (3) BURGESS AND ROSE: J. Am. Chem. Soc. 51, 2127 (1929).
- (4) BURGESS: Unpublished observations.
- (5) HOLDEN AND BURGESS: Unpublished observations.
- (6) MATHEWSON: Z. anorg. Chem. 48, 196 (1906).
- (7) KRAUS AND KURTZ: J. Am. Chem. Soc. 47, 43 (1925).
- (8) BERGSTROM: J. Am. Chem. Soc. 46, 1545 (1924).
- (9) BERGSTROM: J. Am. Chem. Soc. 45, 2788 (1923); 47, 1836 (1925).
- (10) HOVBY AND BURGESS: Unpublished observations.

THE PROPERTIES OF SOLUTIONS OF METALS IN LIQUID AMMONIA

WARREN C. JOHNSON AND ALBERT W. MEYER¹

Department of Chemistry, University of Chicago, Illinois

Received February 6, 1931

Liquid ammonia is an excellent solvent for a large number of different types of substances (1). Although it does not surpass water as a solvent for most inorganic substances, it appears to excel as a medium for organic compounds; in this it resembles alcohol. It exhibits a distinct advantage over water in that it dissolves the alkali and alkaline earth metals without appreciable reaction with them. The fact that reaction does not proceed is undoubtedly due to the extremely low concentration of the amide ions present in liquid ammonia. This condition is substantiated by the low conductivity of pure ammonia and also by the small tendency for ammonolytic reactions to proceed as compared to hydrolytic reactions in water. Substances which are readily hydrolyzed in water are, as a rule, not ammonolyzed in liquid ammonia. Thus it is possible to study the chemical and physical properties of the strongly electropositive elements in a comparatively inert medium possessing a high dielectric constant. In the present discussion we shall confine ourselves to the physical properties of these solutions and shall attempt to give a critical account of the results of the numerous investigations.

SOLUBILITY RELATIONS

Solutions of the alkali metals in liquid ammonia were first investigated by Weyl in 1864 (2). He observed that sodium and potassium were soluble in this medium, producing highly colored solutions. At the time of his studies the radical theory domi-

¹ Present address, The du Pont Ammonia Corporation, Wilmington, Delaware.

nated chemistry. Naturally Weyl regarded these solutions in the light of the theories then prevailing, and concluded that the metals were joined to the nitrogen of ammonia, forming substituted ammonium groups, with the metal taking the place of one of the hydrogen atoms. He did not present any quantitative data to support his conclusions.

A few years later, Seely reported his observations on the solution of alkali metals in liquid ammonia. These results were published during the two years following his report (3, 4, 5). He contended that the metals are in solution in ammonia as such and that they are not united to nitrogen in the form of an ammonium radical. Although he presented no quantitative data to support his views, he described his observations clearly as follows:

Now, the key to the whole subject is the fact that liquid anhydrous ammonia is a solvent, without definite chemical action on the alkali metals. I mean that these metals dissolve in the ammonia as salt dissolves in water—the solid disappears in the liquid, and on evaporating the liquid, the solid reappears in its original form and character. There is no definite atomic action in any such cases; the components of the solution are not changed in their chemical relations to other substances." He describes his apparatus and then continues: "When sodium is subjected in this apparatus to the condensing ammonia, before any ammonia is visibly condensed to the liquid state, it gradually loses its lustre, becomes of a dark hue, and increases in bulk. The solid then appears to become pasty, and at last we have only a homogeneous mobile liquid. During the liquefaction and for a little time after, the mass is of a lustrous, copper-red hue; the condensation of the ammonia and its mingling with the liquid steadily goes on, the liquid is progressively diluted, and passing through a variety of tints by reflected light at last it becomes plainly transparent and of a lively blue by reflected as well as by transmitted light; the liquid now closely resembles a solution of aniline blue or other pure blue dye-stuff. On reversing the process by cooling the ammonia generator, the ammonia gradually evaporates out of the liquid, and the changes observed during the condensation reappear in the reverse order, till at last the sodium is restored to its original bright metallic state. If the evaporation be conducted slowly and quietly, the sodium is left in crystals of the form seen in snow. The formation of the transparent blue liquid, and the restoration of the sodium are stead-

ily progressive; and the repeated and closest scrutiny of the process has failed to reveal the slightest irregularity in its continuity. The inevitable conclusion from such facts is that the blue liquid is a simple solution of sodium in ammonia, not at all complicated or modified by any definite chemical action.

Seely carried out similar experiments with potassium, lithium and rubidium, and arrived at conclusions analogous to those obtained in the case of sodium solutions. He also observed that lithium appears to be less soluble in ammonia than are the other alkali metals. He evidently did not have any of the alkaline earth metals at his disposal, for, if he had, he would undoubtedly have observed their peculiarities. On the other hand he noticed that aluminium, magnesium, thallium, indium, mercury and copper were not soluble in liquid ammonia.

With the exception of some facts on solubilities given by Gore (6, 7) immediately following the work of Seely, no experiments were reported for several years. The subject was finally revived by Moissan and Joannis who carried out many investigations over a period of years. Moissan (8, 8a) and Guntz (9) finally added cesium, calcium, barium and strontium to the list of soluble metals. Cottrell (10), at a much later date, found magnesium to be slightly soluble in ammonia, contrary to the observations of Seely. That the alkaline earth metals present a situation somewhat different from that of the alkali metals was observed by several investigators. This part of the discussion will be considered later in reference to the possibilities of the existence of combinations between the soluble metals and ammonia.

Although most of the earlier workers recognized that these metals are highly soluble in ammonia, relatively few attempts were made to determine the solubilities quantitatively. Ruff and Geisel (11) determined the solubility of lithium, sodium and potassium in liquid ammonia by a unique method, one which was subject to errors of considerable magnitude, but which, nevertheless, through careful and skillful manipulation, gave them fairly consistent and accurate results.

They allowed an amount of ammonia, insufficient to dissolve all the metal, to be added to lithium, sodium and potassium in

small tubes. Some cotton wadding was placed above the mixtures and the upper end of each tube was sealed. The tubes were then agitated for three hours to insure saturation. After this period they were inverted to allow the solution to filter through the wadding. When an amount sufficient for analysis had filtered, the tubes were immediately placed in a Dewar flask containing liquid air, in order to freeze the entire contents. Then the bottom part of each tube was broken off immediately above the saturated solution without allowing the contents to melt. The solution was finally analyzed according to the usual procedures.

They found that the solubility of metallic lithium in ammonia does not appear to change over a temperature range from -80° to 0° , while that of potassium increases with increasing temperature from -100° to 0° . Over the same temperature range the solubility of sodium was found to decrease with increasing temperature. Although the changes in solubility with the temperature are relatively small in all three cases, it is difficult to understand the lack of change in the case of lithium. Since Ruff and Giesel took the average of two series of determinations, the values of which were decidedly at variance with each other, it seems probable that the accuracy of the method would not allow for the detection of small changes in solubility. Their values for these three metals are given in table 1.

A convenient and accurate method for a determination of the solubility of a metal in liquid ammonia is to measure the vapor pressure of the solutions as a function of the concentration of dissolved metal. When ammonia is added to the metal, the resulting solution shows a constant vapor pressure, that of the saturated solution, as long as any undissolved metal remains. The change in the vapor pressure in passing from the saturated solution to those less concentrated in metal is very abrupt (see figure 1). It is possible to obtain several values of the pressure in this region by careful manipulation in the experimental procedure. When this curve is extrapolated to intersect the horizontal line representing the vapor pressure of the saturated solution, a point

is obtained corresponding to the composition of the saturated solution.

Joannis (12) applied this procedure to solutions of sodium and also of potassium at many different temperatures. The results obtained by Joannis are not in accord with those of Ruff and Geisel and of later workers. In some temperature intervals, even the sign is opposite. It would appear that Joannis experienced diffi-

TABLE I

Composition of saturated solutions of alkali metals in liquid ammonia at different temperatures

A. Lithium

TEMPERATURE	0°	25°	-39.4°	-50°	-80°
Kraus and Johnson.....			3.61		
Ruff and Geisel.....	3.93	3.93	3.93	3.93	3.93

B. Sodium

TEMPERATURE	0°	-33.8°	-50°	-70°	-105°
Kraus, Carney and Johnson.....		5.48			
Ruff and Geisel.....	5.87	5.48	5.39	5.20	4.98
Kraus and Lucasse.....		5.37			
Johnson and Meyer.....	5.79				

C. Potassium

TEMPERATURE	0°	-33.5°	-50°		-100°
Kraus and Lucasse.....		4.87			
Ruff and Geisel.....	4.74		4.79		
Johnson and Meyer.....	4.68	4.95	5.05		4.82

culty in establishing equilibrium conditions and in maintaining constant temperature during the course of a series of determinations. It is quite doubtful that the temperatures were accurately known, since the boiling point of liquid ammonia was in error by at least 5°. The changes in vapor pressure of these solutions are very sensitive to small temperature changes.

Applying this procedure under conditions of equilibrium and controlled temperature, Kraus and Johnson (13), Kraus, Carney

and Johnson (14), and Johnson and Meyer (15, 16) have determined the composition of saturated solutions of lithium, sodium and potassium at several temperatures. These results are given in table 1, and are expressed in terms of gram-molecules of ammonia per gram-atom of metal.

The values obtained by Kraus and Lucasse (17) are the result of resistance measurements. They noticed a decided change in

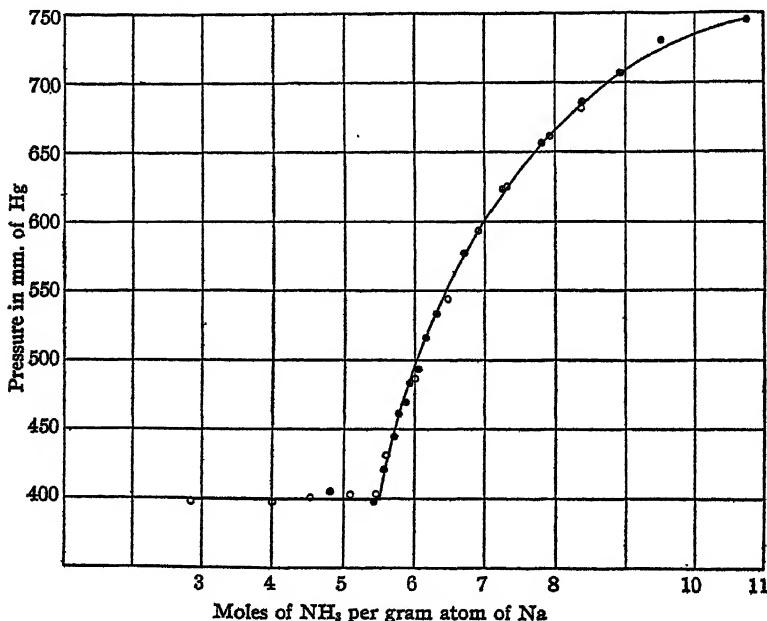


FIG. 1. THE VAPOR PRESSURE OF SOLUTIONS OF SODIUM IN LIQUID AMMONIA AT -33°

the resistance of these solutions in passing from a saturated solution to those more dilute in metal. The point at which such a break occurs is identical with the composition of the saturated solution. Ruff and Zedner (18) give additional solubility data at temperatures ranging from -115° to $+20^{\circ}$.

Kraus (19) has taken the values for sodium secured from conductivity measurements and those of Ruff and Geisel to give the general form of the solubility curves over a considerable range

of temperature. He also presents evidence to show that at low temperatures the sodium solutions separate into two liquid phases. Ruff and Zedner (18) determined the composition of these liquid phases in equilibrium with each other and with vapor, by noting the temperature at which the two-phase system disappeared. According to their results, this temperature lies at about -45° and at a composition of approximately two atom per cent of sodium. This value is not in accord with the vapor pressure curves of Kraus (20) which, at higher temperatures, show an inflection point at about 3.5 atom per cent of sodium. Later, Kraus and Lucasse (21) determined the composition of these two liquid phases at low temperatures. They found that when the temperature reaches that of the three-phase system the electrical resistance of the solution undergoes an abrupt change as the second liquid phase appears. This would necessarily be the case since the compositions of the two phases differ appreciably from that of the single liquid phase. The critical solution temperature was found to be -41.6° with a composition of 4.15 atom per cent of sodium. As the temperature is lowered one phase becomes richer in sodium while the other liquid phase decreases in sodium content. It is remarkable, however, that the critical phase appears at such a low concentration of dissolved sodium. Even at -60° , the compositions of the two liquid phases are only 1.7 and 8.3 atom per cent of sodium respectively.

COMBINATIONS BETWEEN METALS AND AMMONIA

Weyl (2) looked upon the solutions of metals in ammonia as being true compounds, in which the metal is directly connected to nitrogen as hydrogen is in the ammonium group. The term "metal ammonium" was used to represent such a combination. Seely deduced from his observations that the metals are actually in solution as individuals without combination with the solvent. Neither investigator presented evidence of a quantitative nature to support his view. Joannis (22, 23), at a later date, revived the ammonium theory and carried out a large number of experiments to determine the vapor pressures of the solutions and the molecular weights of the dissolved metals (24, 25, 26, 27). He believed his work

showed that the molecules in solution contain two atoms of sodium or two of potassium. Among others, Moissan (8, 28, 29, 30, 31, 32) applied the method of Joannis with similar results. Thus the term "metal ammonium" was used for several years by a number of workers in the field and has without justification found some following even to the present day. After the careful experiments of Ruff and Geisel (11) and Kraus (33) had appeared, experiments which showed that, from the standpoint of the phase rule, no solid constituent containing ammonia could exist, the term "metal ammonium" has been to a large extent discarded.

The argument to show the non-existence of alkali metal-ammonia combinations appears simple when one examines carefully the form of the vapor pressure curves for these solutions. In figure 1, the horizontal line represents the pressure of saturated solutions of sodium in liquid ammonia at -33.5° . This value is about 400 mm. of mercury. The composition of the saturated solution is indicated at the point of intersection of the horizontal line with the vertical line representing pressures for the more dilute solutions. As the solutions become more dilute in metal the vapor pressure increases very rapidly and assumes a fairly constant value after having traversed a comparatively short range of concentration. Joannis argued that the decomposition pressure of sodium ammonium is the same as the vapor pressure of its saturated solution. In such a case a new solid phase would appear in the equilibrium, and, according to the phase rule, the condition could be realized only at one definite temperature since the system would be non-variant. Roozeboom (34) pointed out that Joannis must have carried out his experiments at the temperature of the non-variant equilibrium. However, measurements at other temperatures give the same type of curve to show that the solid phase separating from solution is identical with the phase of sodium metal. Measurements at very low temperatures, about -70° , show no change in the form of the vapor pressure curve. At these temperatures the ammonia may be easily removed from saturated solutions leaving metal only.

More recently Benoit (35) has published the results of an investigation of the phase relations in the system lithium-ammonia.

Working at -38.5° he found three regions within which the vapor pressure of the solutions appears to be independent of the composition of the system. He concluded from this that a lithium ammonium is present with the composition corresponding to the formula $[\text{Li}(\text{NH}_3)_4]_2$. Repetition of this work shows (13) the nonexistence of a region of constant pressure other than that for saturated solutions. It appears that Benoit did not establish

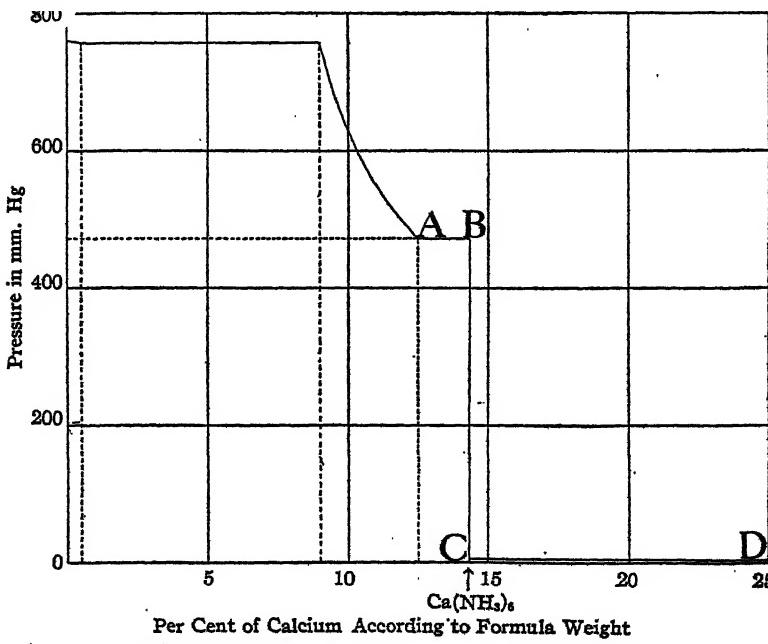


FIG. 2. THE SYSTEM CALCIUM-AMMONIA AT -32.5°

true equilibrium conditions. It is impossible to obtain consistent results for the vapor pressure of these solutions unless they are strongly agitated throughout the series of measurements. It is also necessary to approach the equilibria from both sides to be certain of the conditions. Benoit gives no description of the method he employed for maintaining equilibrium. According to the data existing for the vapor pressure of pure ammonia, Benoit evidently worked at a temperature that was somewhat higher than he records.

Thus all attempts to show the existence of combinations between the alkali metals and ammonia have failed completely. Even though there were no evidence to show this, it would be exceedingly difficult to conceive of a highly electropositive element taking the place of a hydrogen atom in the ammonium group.

An examination of solutions of the alkaline earth metals in liquid ammonia presents a somewhat different situation. A break in the vapor pressure curves is found in which the pressure is independent of the composition of the solution throughout a definite range of concentration. Kraus (33), (see figure 2), has shown that calcium forms a solid phase in which six molecules of ammonia are associated with an atom of the metal (8a). This compound, however, is to be regarded as an ammoniate, analogous to the ammoniated salts such as $\text{CaCl}_2 \cdot 6\text{NH}_3$, etc., rather than as a metal ammonium. It possesses the appearance of a metal and appears to have all the characteristic properties of metals. Mentrel (36) and Roederer (37) have established the existence of hexammoniates of barium and strontium. No similar combination has been noted for magnesium. The alkaline earth metal ammoniates have also been prepared and studied by Biltz and Huttig (54).

MOLECULAR WEIGHTS

The first molecular weight determinations of the metals in liquid ammonia were carried out by Joannis (12, 27). Working with solutions of sodium and potassium, he determined the change in the vapor pressure of the solvent as a function of the composition of the solution. Then, by means of Raoult's law, he calculated the molecular weight from the data. Some of his results are undoubtedly in error due to the fact that he assumed an incorrect value for the boiling point of liquid ammonia. He observed the boiling point of the solutions under atmospheric pressure, employing a value of -38.2° for the boiling point of pure ammonia. Several independent determinations have since shown the boiling point of ammonia to be about -33.5° . Joannis also applied the law of Raoult to solutions highly concentrated in metal; the most dilute solutions measured were about 3.70 *N*

with respect to sodium or potassium. It is evident that at such high concentrations the application of Raoult's law would have no meaning, particularly in systems of this type which appear to deviate from the law to a greater extent than do typical solutes. Thus one is not justified in using the results of molecular weight experiments in highly concentrated solutions to determine the state of the molecular species, whether it is postulated as a representation of solvent combination or of more simple species. This is one reason why similar arguments proposed by Joannis for the state of aggregation of the so-called metal ammoniums bear little weight. This fact was pointed out by Kraus and Ruff and Geisel (see preceding discussion).

TABLE 2
Apparent molecular weight of sodium in ammonia at different concentrations

CONCENTRATION	APPARENT MOLECULAR WEIGHT	CONCENTRATION	APPARENT MOLECULAR WEIGHT
2.903	32.23	0.3665	25.31
1.841	30.70	0.3587	25.27
1.220	29.06	0.2689	23.53
0.9910	28.80	0.2516	23.43
0.9038	28.46	0.2261	23.41
0.5614	26.39	0.1565	21.62
0.5558	26.47	0.1519	21.58
0.4104	25.36		

Franklin and Kraus (38) determined the molecular weight of sodium and also of lithium in liquid ammonia by the boiling point method. The concentration of the sodium solution ranged from 0.3 N to 1.3 N and the *apparent* molecular weight showed an increase from 36 to 59. Similar results were obtained with lithium. The boiling point method was recognized as not admitting of a high degree of accuracy owing to several factors. In dilute solutions, particularly at the temperature of boiling ammonia, the metals exhibit some tendency to react with the solvent to form the metal amides, which in turn act as catalysts for this reaction. Small amounts of impurities in the metals such as oxides, iron, etc. appear to have a similar effect.

With careful manipulation and exclusion of impurities Kraus (20) devised a method to determine pressure changes resulting in the addition of sodium to liquid ammonia. He was able to secure an accuracy of about 1 per cent when the total pressure change was as small as 10 mm. The measurements were carried out at 15° and the dilutions of the solutions varied from 1 to 13 liters per gram-atom of sodium. He demonstrated that Raoult's law could not be applied to solutions more concentrated in metal than 0.1 *N*. The results are exceedingly interesting in that in dilute solutions the apparent molecular weight is less than 23, while in the more concentrated solutions this value becomes markedly greater than 23. (See table 2.) A value of 23 was obtained at concentrations in the neighborhood of 0.1 *N*. An examination of the vapor pressure curves (figure 1) shows that in the more concentrated solutions the change in the vapor pressure is exceedingly large in relation to the change in composition. Deviations from Raoult's law would be expected to be very great in these regions. It would be desirable to have additional data in solutions more dilute in metal than have thus far been studied, but the difficulties in manipulation and control limit the methods applicable to such systems. Ruff and Zedner (18) also measured the change in the boiling point of solutions of sodium, potassium and lithium. Their results are in agreement with those of Kraus in that, in fairly dilute solutions, the apparent molecular weight is too small and in concentrated solutions it is too great. This corresponds to a deviation in the vapor pressure first on one side and then on the other side of the theoretical curve.

The fact that the molecular weight values obtained in the more dilute solutions are less than 23 is an indication of dissociation. It is known that these solutions are excellent conductors of the electric current and exhibit electrolytic properties in dilute solutions. Since the alkali metals behave as binary electrolytes in this medium, very dilute solutions should show a value of the molecular weight approaching one-half that of the atomic weight of sodium. It may be possible to demonstrate this at a later date.

ELECTRICAL PROPERTIES

A study of the nature of the conduction process in solutions of metals in liquid ammonia has occupied the attention of relatively few workers. On the other hand, the results available to date have attracted considerable interest, since these systems represent a metal dissolved in a non-metallic medium in which a continuous change of one type to the other is exemplified. These results bear an important relation to the metallic state of matter and the process of the passage of electricity in metals.

From the earlier studies it was observed that liquid ammonia is capable of dissolving considerable amounts of the alkali metals to form stable solutions. Studies with solutions of acids, bases and salts in ammonia showed in the pioneering work that the solutions possessed the ability to conduct the electric current. They also demonstrated ammonia to be an excellent dissociating medium. That solutions of the metals should conduct the current with great facility, even to a greater extent than do the typical electrolytes in this solvent, probably would have been beyond the highest expectations.

Cady (39) was the first to make any observations in this direction. He found solutions of sodium in ammonia to be excellent conductors, with the order of magnitude of the conductance being markedly greater than that of typical electrolytes in the same solvent. He also noticed that in concentrated solutions no material effects appear to accompany the passage of electricity, while in dilute solutions there is an increase in the concentration of metal at the cathode. The problem was later studied systematically and thoroughly by Kraus, whose results are reported in a series of papers appearing in the period of 1907-1921 (19, 20, 33, 40, 41, 42, 43). Gibson and Argo (44, 45) and Gibson and Phipps (46) added materially to the problem.

Shortly following, the work of Cady, Franklin and Kraus (47) confirmed the fact that ammonia solutions of the metals conduct electricity without polarization at the electrodes.

The first experiments of Kraus were carried out for the purpose of determining whether or not the conduction process is ionic in nature. This was done by observing the migration phenomena

in solutions containing only the metals and ammonia. Since the metals exhibit characteristic colors in solution (48), and in dilute solutions as small an amount as 1/40000 *N* of metal can be easily detected, it is possible to follow changes visually. Thus, a small amount of sodium was dissolved in liquid ammonia in a cell designed for the experiment. On the application of a potential of 110 volts, the color about the anode at once began to grow less intense, while the color in the neighborhood of the cathode deepened perceptibly. The color of the solution above the cathode did not change appreciably and thus served as a standard in determining the colors in other parts of the cell. At the end of half an hour, the cathode region became quite opaque while the anode electrode was visible. No evidence was found of an anode product. When the solution about the anode had become quite dilute, the current was reversed; the clear space at once became broadened in the form of a wedge with the apex at the bottom of the electrode. The blue solution was now in contact with the bottom of the cathode electrode. This effect appeared to be more pronounced in dilute than in concentrated solutions. When the current was measured as a function of the time, it was observed to decrease quite rapidly, and to follow the color changes taking place in the neighborhood of the electrodes.

Kraus (40) interpreted these results as indicative of an ionic conduction process, since a transfer of matter was found to take place with the electric current. The experiments showed sodium to be a constituent of the positive ion, while the negative ion was postulated as being identical with the negative electron which is in equilibrium with the positive sodium ion and sodium metal as follows:



Kraus states:

Aside from the important fact that the conduction process in metal solutions is an ionic one, which follows at once from the preceding experiments, it remains to examine more closely the phenomena taking place in the neighborhood of the electrodes. We will first consider the anode,

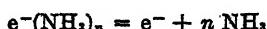
where the phenomenon is less complex. There can be no question that a gaseous product is not evolved here. The solution being transparent, the formation of minute bubbles of gas would readily be observed. At the same time the current is by no means inconsiderable, rising to as much as 0.05 ampere. A current of 10 milliamperes generates a cubic millimeter of hydrogen per second. A gas, therefore, is not evolved. Moreover, no electrolytic product results at the anode by interaction between the negative carrier and the solvent, or the electrode, for in that case we should always have left in the anode space a certain amount of ionic material. In these solutions, however, the resistance increases to something like a million ohms per centimeter in a comparatively short time. The only remaining possibility then, is that the negative ion reacts at the anode to form a soluble neutral molecule. As the electrodes are not attacked, such reaction could involve only the solvent itself.

It is difficult to imagine a reaction taking place between ammonia and the anion Y^- to form a soluble, non-electrolytic substance at the anode, unless ammonia itself constitutes the negative ion Y^- . In that case, of course, the ammonia would be discharged at the anode and simply add itself to the remaining solvent. We must not forget that the ion Y^- is formed by dissolved neutral metal atoms in neutral ammonia and that this solution permits of reversible separation of its components. If the metal forms the positive ion Na^+ , as we have shown, then the negative charge only can be associated with ammonia. According to our present views of matter and electricity, negative electricity consists of discrete charges called electrons, whose mass is about 1/1000 that of the hydrogen ion. If we assume tentatively that the metal atoms lose electrons when they dissolve in ammonia, then the solvent may of course take up the negative electrons in some way.

Making use of these results, the above expression may be re-written as follows:



where Na^+ represents the normal sodium ion and e^- the electron. They also indicate that the electron cannot be free as such in ammonia, but that it is undoubtedly associated with the solvent molecules. Thus, the ammoniated electron is in equilibrium with ammonia and the free electron:



Kraus then carried out some migration experiments with solutions of potassium in potassium amide dissolved in liquid ammonia and was successful in showing that the positive ion obtained by dissolving metallic potassium in ammonia is identical with the positive ion resulting from the dissociation of potassium amide in the same medium.

Measurements of the electromotive force of concentration cells of solutions of sodium in ammonia were made by the same investigator at a later date (41). Transference numbers for several

TABLE 3

E.M.F. of concentration cells and values of n and $(1-n)/n$ for solutions of sodium in liquid ammonia

C_a	$E \times 10^8$	n	$(1-n)/n$
0.870	0.080	0.00359	277.6
0.732	0.328	0.0109	90.6
0.335	0.620	0.0231	41.2
0.164	0.72	0.0291	33.4
0.081	0.86	0.0336	28.8
0.040	1.07	0.0385	25.0
0.020	1.38	0.0575	16.4
0.010	1.80	0.0704	13.2
0.005	2.60	0.0980	9.2
0.0024	3.40	0.125	7.0

different concentrations were calculated from the results. The ratio of the fraction of the current carried by the two ions was found to approach a limiting value of approximately 7 in dilute solutions, and to increase to a value of 280 at a concentration of a little less than normal. (See table 3.) With a constant speed assumed for the positive sodium ion, the mean speed of the negative carrier increases in the neighborhood of 40 times between 0.001 and 1.0 N (53). Previous transference experiments with salt solutions give a value of 130 for the equivalent conductance of the sodium ion. On this basis, the value for the negative carrier is 910, and in dilute solutions of the metals the equivalent conductance should approach a value of 1040. This value is in good agreement with that obtained from conductivity data which were published several years later.

Kraus interprets these results on the assumption that the ions Na^+ and e^- exist in solution with the negative carrier surrounded by an envelope of ammonia molecules. The extent of ammoniation of these carriers is postulated as the determining factor in their velocity. He explains his results at higher concentrations in that some of the electrons are freed from the ammonia envelope and consequently move with a speed comparable to that of electrons in metals. This accounts for the rapid increase of the transference number of the negative carrier at high concentrations. The number of free electrons was found to increase as the proportion of metal to ammonia increases, that is, on the basis of transference.

Studies of the conductance of metal solutions have shown that the process is essentially similar to that in aqueous solutions and that our familiar ideas of conduction and dissociation can be extended to ammonia solutions. On dilution, the molecular conductivity approaches a maximum value, just as does that of aqueous solutions, to show that at infinite dilution there is complete ionization. It is perhaps significant, however, that ammonia solutions of strong electrolytes conform more nearly to the law of mass action at low concentrations than do similar water solutions. A rather remarkable fact is that the ions travel faster in ammonia than in water. One cause of this may be the low viscosity of liquid ammonia. Because ammonia is less viscous and is a weaker dissociating agent than water, dilute ammonia solutions are better conductors than dilute aqueous solutions, while water solutions excel when concentrated.

Kraus (43) made an extensive study in which he measured the electrical conductances of solutions of sodium, potassium and lithium over a wide range of concentration. Using small platinum electrodes in his cells, so as to reduce the reaction between metal and ammonia to a minimum, and employing an electromagnetic stirrer to maintain the solutions in equilibrium, he was successful in securing reproducible results. In figure 3, the results of these measurements are plotted for the three alkali metals mentioned above. The equivalent conductance A is plotted against

the logarithm of the dilution, V . (Since the densities of these solutions were not known at the time of the conductance measurements, the dilution is expressed in terms of 1000 cc. of ammonia). Due to the exceedingly high values for the conductivity at high concentrations, it is not possible to include them on a single graph with the values at low concentrations.

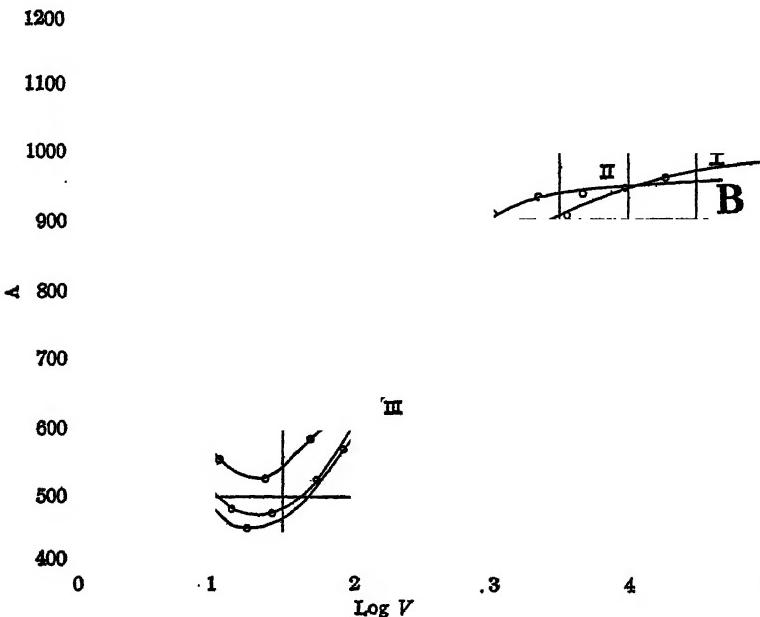


FIG. 3. THE CONDUCTIVITIES OF SOLUTIONS OF SODIUM (I), OF POTASSIUM (II), AND OF LITHIUM (III) IN LIQUID AMMONIA AT -33°

In the dilute regions it is at once noticed that as the dilution increases the equivalent conductance increases and approaches a constant value. The form of the curve in this region, from A to B, is similar to that of ordinary electrolytes dissolved in liquid ammonia. When C is plotted against $1/\Delta$, where C represents the concentration ($C = 1/V$), a straight line is obtained for the values at low concentrations, which gives on extrapolation a value of 1016 for the equivalent conductance at infinite dilution. (See table 4.) Thus the value of the mass action constant is

77.27×10^{-4} . In the most dilute solutions, some of the points are somewhat irregular owing to the reaction of metal with ammonia. It is interesting to note, however, that the limiting value of the conductance which is approached at low concentrations is very much higher than it is in the case of typical salts dissolved in ammonia. Some salt solutions approach a value as high as 340, which is only about one-third of that reached in the metal solutions.

As the concentration of the metal in solution is increased, the conductivity is noticed to drop off considerably and pass through

TABLE 4
The conductance of sodium in liquid ammonia at -33.5°

V	A	V	A
0.5047	82490.0	13.86	478.3
0.6005	44100.0	30.40	478.5
0.6941	23350.0	65.60	540.3
0.7861	12350.0	146.0	650.3
0.8778	7224.0	318.6	773.4
0.9570	4700.0	690.1	869.4
1.038	3228.0	1551.0	956.6
1.239	2017.0	3479.0	988.6
2.798	749.4	7651.0	1009.0
6.305	554.7	17260.0	1016.0
		37880.0	1034.0

a minimum at a concentration of about 0.05 N. Further increased concentration of metal causes the conductance to rise rapidly to values of almost unimaginable dimensions. For a concentration of approximately normal, the equivalent conductance rises to a value of approximately 3000, while at 2 N a value of 80,000 is reached for sodium solutions. Kraus and Lucasse (17) have determined values for saturated solutions of sodium and potassium and have found the specific conductivities to be 0.5047×10^4 and 0.4569×10^4 , respectively. The specific conductance of mercury is 1.063×10^4 , which value is only a little more than twice that of a saturated solution of sodium in ammonia and only 6 times that of a solution 2 N in sodium. If a comparison is made

on the basis of atomic conductances instead of specific conductances, a better correlation is noted. For example, the atomic conductance of iron is 0.8031×10^6 , of strontium, 0.7194×10^6 , and of mercury, 0.1564×10^6 , while that of a saturated solution of sodium in liquid ammonia is 0.6720×10^6 , if the assumption is made that the density of the solution is equal to that of the pure solvent. Since the density is now known to be considerably less than that of the pure solvent, the above value calculated for sodium is less than the true value. It is quite apparent that such large conductance values can not be attributed to electrolytic conduction.

If a value of 1016 is taken for the equivalent conductance at infinite dilution, and a value of 130 for the equivalent conductance of the sodium ion, the equivalent conductance of the negative carrier must be 886. This value is in good agreement with that obtained from the results of electromotive force measurements in dilute solutions. If the negative carrier at low concentrations is the electron, it immediately follows that it must be assumed to be associated with solvent molecules to account for its low conductivity. If it were free to move without any solvent disturbances or combinations, it would readily move much faster than is indicated by the results of conductivity measurements. Jaffe (49) has measured the speed of the electron produced by radiations in hexane and found a value of 4.17×10^{-4} centimeters per volt second. On the basis of the value of the conductance of the electron in ammonia solutions the speed of this negative carrier is 91.7×10^{-4} centimeters per second. So the electron appears to move much faster in ammonia than in hexane. Since the dielectric constant of ammonia is much higher than that of hexane, it would be expected that the electron would be less associated with solvent molecules in ammonia than in hexane. That the combination is not a very stable one is seen in the high conductance values at high concentrations, where a large part of the negative carriers must be free to move without the constraints of the solvent, much as the electron moves in metals. The ammoniated electron may be looked upon as possessing a stability of

the order of magnitude found in the ammoniation or hydration of other ions.

Kraus has summed up the results of his studies of the conductivities of solutions of metals in liquid ammonia (43):

These solutions, therefore, constitute a connecting link between metallic and electrolytic conductors. In dilute solution the process is, at least in part, electrolytic. A portion of the current is carried by the positive carriers as they appear in solutions of the common salts. The negative carrier is chemically uncombined but is associated with one or more molecules of the solvent. These carriers are identical for solutions of all metals, and, when the discharge occurs at the anode, the only material process which takes place is that a portion of the solvent is left behind in the immediate neighborhood of this electrode. As the concentration of the solution increases, the nature of the phenomenon changes only insofar as the combination of the negative carrier with ammonia is effected. At the higher concentrations, the negative carriers are free from association with ammonia molecules to a greater and greater extent. And, since under these conditions, the negative carrier is associated with no matter of atomic dimensions, it follows that all the material effects cease so far as these carriers are concerned. It is not to be understood that a given carrier is free from association with the solvent molecules for any considerable period of time. Obviously, an equilibrium must exist between the free carriers and the combined carriers and ammonia, which results in a constant interchange between the free and bound carriers. During the interval over which these carriers are free from the solvent molecules, they conduct just as they do in metals. As the concentration is further increased, the number of free carriers increases. It is evident that their number in the more dilute solution, for example in the neighborhood of normal, must be relatively small, since at the higher concentrations the equivalent conductance reaches values some one hundred times as great as that at normal concentration. It is not possible to determine the actual number of carriers in the more concentrated solutions. In the more dilute solutions, however, it appears that the number of carriers decreases with increasing concentration, just as it does in the case of normal electrolytes in ammonia.

There is nothing to distinguish the more concentrated solutions from actual metallic substances. It may be concluded, therefore, that the process of conduction in the case of the ordinary metals is effected by

means of the same negative carrier. Since this carrier is negatively charged and has sub-atomic dimensions, we may conclude that it is identical with the negative electron as it appears in radioactive and other phenomena.

Gibson and Phipps (46) measured the conductance of solutions of sodium and also of potassium in liquid ammonia, and of cesium, cesium iodide and potassium in methylamine at several different temperatures. Their results in liquid ammonia correspond well with the measurements of Kraus. They used a constant volume method, and it was possible for them to express the concentration of the solutions in terms of volume normal. Since the densities of these solutions at high concentrations are exceedingly abnormal, deviations in the conductance as determined by the two methods are more marked; however, in the more dilute solutions, the agreement is good. Their results in methylamine solutions are very interesting. They found the metals to be less ionized in methylamine, with the minimum in the conductivity occurring at a higher concentration than in ammonia. The properties of the metals in solvents related to liquid ammonia constitute another subject which cannot be discussed here at length.

The resistance-temperature coefficient of dilute solutions of sodium in ammonia has been shown by Kraus (43) to be positive and, in the neighborhood of the boiling point of liquid ammonia, to have a value of about 2.5 per cent per degree in terms of the resistance at the boiling point of ammonia. In the more concentrated solutions, similar measurements were carried out by Kraus and Lucasse (21) from the saturation point to a dilution of 5.02 liters and at temperatures from -33.5° to -60° . The temperature coefficient of a saturated solution was found to have a value of 0.066 per cent. With decreasing concentration, the coefficient increases markedly, passing through a maximum value of about 3.6 per cent at a dilution of 1.1 liters, and thereafter decreases to a value of about 1.55 at dilutions above 4.0 liters. In the case of potassium solutions (50) similar results were obtained. Gibson and Phipps measured the temperature coefficients of resistance for sodium and potassium solutions with corresponding results. They calculated the differential tempera-

ture coefficient, which was found to be independent of the temperature. In other words, the conductance is an exponential function of the temperature at all dilutions.

That the negative carrier is the same in all solutions of metals in ammonia has been well brought out by Gibson and Argo (44, 45) in their photometric measurements. They determined the absorption of lithium, sodium, potassium, magnesium and calcium solutions at low concentrations and obtained practically identical results. The absorption curves for all these metal solutions were found to be the same. This result must necessarily lead to the assumption that in these solutions there is present a particle that is common to all. Since the experiments were made in dilute solutions, in which there are no free electrons, it would appear that the color and the absorption is due to complexes being formed between the electron and solvent molecules. It would be highly interesting to investigate the absorption of these solutions at higher concentrations where there are present negative carriers that are not associated with molecules of ammonia.

DENSITIES

Seely (4) observed in his first qualitative experiments that an increase in volume results when ammonia is added to the alkali metals. Kraus and Lucasse (17, 21) noticed this abnormal change and made some calculations based on the volume of their conductivity cells. They calculated the density of a saturated solution of sodium in liquid ammonia to be 0.54, and that of a saturated potassium solution, 0.632. The density of pure liquid ammonia at -33.8° is 0.6824 (51). Kraus, Carney and Johnson have measured the density of sodium solutions over a fairly wide range of concentration at -33.8° . A value of 0.5782 was found for the density of the saturated solution. This means that if a saturated solution of sodium in ammonia is prepared, one in which for every gram-atom of sodium (23 grams) there is present approximately 93 grams (5.5 moles) of ammonia the volume of the mixture is markedly greater than the sum of the volumes of the two constituents. Twenty-three grams of sodium occupy about 23 cc. and 93 grams of liquid ammonia about 135 cc. at

-33°. If no change in volume were to take place when the sodium is dissolved, the total volume of the solution would be about 160 cc. However, calculations from the measured density as given above show that the total volume is slightly more than 200 cc., which represents a volume increase of 41 cc. or approximately 25 per cent. No other system shows a volume change of such magnitude.

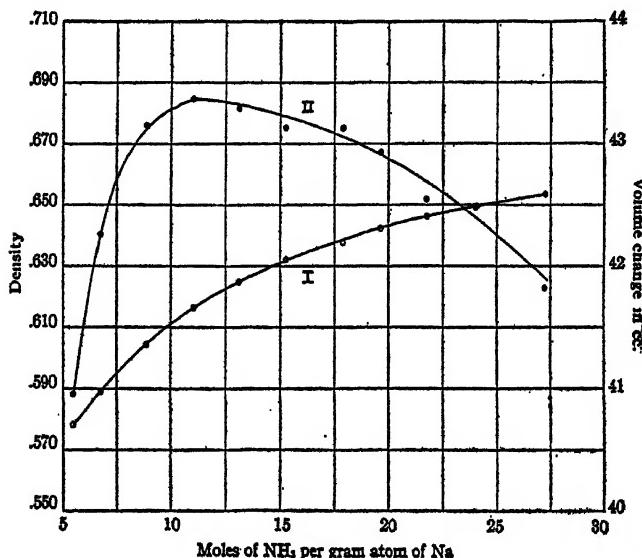


FIG. 4. THE DENSITY AND VOLUME CHANGE FOR SOLUTIONS OF SODIUM IN LIQUID AMMONIA AT -33.8°

The results for sodium solutions are plotted in figure 4. Curve I represents the density as a function of the concentration expressed in terms of gram-molecules of ammonia per gram-atom of sodium. Curve II gives the volume change accompanying the formation of the solutions from their components. The density is seen to increase steadily with increasing dilution and to approach the value for pure ammonia at low concentrations. The volume change for a saturated solution is about 41 cc. per gram-atom of sodium. With decreasing concentration, the vol-

ume change first increases, reaching a maximum of 43.4 cc. per gram-atom of sodium at a concentration of 11.25 gram-molecules of ammonia per gram-atom of sodium. At still lower concentrations the value of the volume change decreases slowly.

Johnson and Meyer (16) have made some measurements of the densities of sodium and potassium solutions. In the case of the sodium solutions, the density was determined at a concentration as low as 64.14 gram-molecules of ammonia per gram-atom of sodium, and as high as that of the saturated solution. These values agree with those shown in the figure, while the values at low concentrations give a regular curve. When it is extrapolated to infinite dilution, a value for the density of pure ammonia is obtained which agrees with that given by Cragoe and Harper. The density of a saturated solution of potassium in liquid ammonia is somewhat higher than that for sodium. The value at -33.2° is 0.6282. This is in good agreement with the value calculated by Kraus and Lucasse. The form of the density curve for potassium is the same as that for sodium. The volume change curve is also of the same type; a value of 27.34 cc. per gram-atom of potassium is found for the saturated solution. As the solution becomes more dilute in metal, the volume change increases to a maximum value of 29.50 at a concentration of about 13.0 gram-molecules of ammonia per gram-atom of potassium; then, in the more dilute regions, the volume change gradually decreases.

If one examines the densities of solutions of ordinary salts in liquid ammonia (16), no abnormalities are found. A solution of sodium bromide in ammonia has a density greater than that of pure ammonia, and as the solution is diluted the density decreases and approaches that of ammonia. Thus, the increase in volume noted in the case of the metal solutions can not be attributed to the metal ions; it must be due to the negative constituent, the electron associated with solvent molecules. The envelope of ammonia molecules associated with the electron must possess a considerable volume to account for these results. The partial molal volumes have been calculated (16) for sodium and potassium in liquid ammonia as well as for some of the salts

in this medium. It is quite probable that when sufficient data of this nature have been collected, so that a comparison may be made of the metal solutions with the salt solutions at very low concentrations, more may be said about the nature of this negative constituent. It should be possible to obtain a relative value for the volume occupied by the ammoniated electron.

ACTIVITIES

An examination of the form of the vapor pressure curves (figure 1) for solutions of metals in liquid ammonia reveals a tremendous change in the vapor pressure with composition, particularly in the more concentrated regions. In order to apply the vapor pressure results for a comparison of the various thermodynamic functions for different metal solutions, the activities of metals in liquid ammonia have been calculated (16). For this purpose the Duhem equation is applied in the following form:

$$d \log a_2 = - N_1/N_2 d \log a_1$$

If the mole ratio N_1/N_2 is plotted as ordinates against $10 + \log a_1$ as abscissae, then, upon integrating between the limits a_s and a_m , where a_s is the activity of the saturated solution which, as a standard, may arbitrarily be set equal to unity, and a_m is the activity at any molality m , the following is obtained:

$$\log a_m/a_s = - \text{Area under the curve between } a_s \text{ and } a_m$$

In this calculation a_s , the activity of the solvent, is set equal to p_1/p_1^0 , where p_1 is the vapor pressure of the solution at any given temperature, and p_1^0 is the vapor pressure of the pure solvent at the same temperature. Such calculations of a_2 , the activity of the metal, have been carried out for lithium, sodium and potassium in liquid ammonia at different temperatures. With the value of a_s taken as 1.0 for the saturated solution in each case, calculations show it to be greatly reduced as the concentration of dissolved metal decreases. The activity of potassium changes about 25,000 times at 0° as the concentration changes only 2.571 times, and for lithium the change is about 3×10^9 for a change in

concentration of about 4.0 times (temperature of -39.4°). The large change here is due to the fact that the vapor pressure changes from 4 mm. to 500 mm. over a very small range of concentration. The activity change with concentration in the case of sodium is less than that found for potassium. Brönsted (52) gives values for sulfuric acid in which the change in activity is approximately 2500 times for a concentration change of 17 times.

When vapor pressure data are available for these solutions in the more dilute regions, it would be desirable to calculate the activities on the basis of a 1 *N* solution as the standard state; thus all calculations might be placed on a comparable basis. Then a comparison of the activities of the metals in ammonia could be made with those of salts in the same solvent under comparable conditions. Such a comparison would undoubtedly lead to interesting results.

PHOTOELECTRIC PROPERTIES

A preliminary experiment has been carried out by Kraus (43) to determine whether or not the solutions of metals in ammonia exhibit photoelectric activity. He introduced 0.25 gram of lithium into a tube with a platinum wire sealed in at the bottom to make contact with the metal solution. An aluminum wire was fixed about 3.5 centimeters above the surface of the metal and connected with an electrometer. When the metal dissolved in the ammonia, a photoelectric activity was immediately observed. With a 16-candle power lamp held about a foot from the metal, the electroscope was found to discharge in 5 seconds. It was also observed that the discharge took place with negative as well as positive charge of the electrometer system. This action is explained by the fact that, owing to the presence of the platinum wire in the solution, decomposition takes place rapidly and particles of the solution are projected on the aluminum wire. It was observed that the photoelectric effect is not due to free metal since, in the experiments, the vapor pressure of the solution was much above that required of a saturated solution. The effect is therefore due to the solution of lithium in ammonia.

REFERENCES

- (1) FRANKLIN AND KRAUS: Am. Chem. J. **21**, 8 (1899).
- (2) WEYL: Ann. Physik **121**, 601 (1864).
- (3) SEELEY: Chem. News **22**, 117 (1870).
- (4) SEELEY: Chem. News **23**, 169 (1871).
- (5) SEELEY: J. Franklin Inst. **61**, 110 (1871).
- (6) GORE: Proc. Roy. Soc. (London) **20**, 441 (1872).
- (7) GORE: Phil. Mag. **44**, 315 (1873).
- (8) MOISSAN: Compt. rend. **136**, 177 (1903).
- (8a) MOISSAN: Compt. rend. **127**, 685 (1898).
- (9) GUNTZ: Compt. rend. **133**, 872 (1901); **133**, 1209 (1901).
- (10) COTTERELL: J. Phys. Chem. **18**, 85 (1914).
- (11) RUFF AND GEISEL: Ber. **39**, 831 (1906).
- (12) JOANNIS: Ann. chim. (8) **7**, 5 (1906).
- (13) KRAUS AND JOHNSON: J. Am. Chem. Soc. **47**, 725 (1925).
- (14) KRAUS, CARNEY AND JOHNSON: J. Am. Chem. Soc. **49**, 2206 (1927).
- (15) JOHNSON AND MEYER: J. Phys. Chem. **33**, 1922 (1929).
- (16) JOHNSON AND MEYER: Unpublished results.
- (17) KRAUS AND LUCASSE: J. Am. Chem. Soc. **43**, 2529 (1921).
- (18) RUFF AND ZEDNER: Ber. **41**, 1948 (1908).
- (19) KRAUS: J. Am. Chem. Soc. **29**, 1557 (1907).
- (20) KRAUS: J. Am. Chem. Soc. **30**, 1197 (1908).
- (21) KRAUS AND LUCASSE: J. Am. Chem. Soc. **44**, 1949 (1922).
- (22) JOANNIS: Compt. rend. **112**, 392 (1891).
- (23) JOANNIS: Compt. rend. **113**, 795 (1891).
- (24) JOANNIS: Compt. rend. **109**, 900 (1889).
- (25) JOANNIS: Compt. rend. **109**, 965 (1899).
- (26) JOANNIS: Compt. rend. **110**, 238 (1890).
- (27) JOANNIS: Compt. rend. **115**, 820 (1892).
- (28) MOISSAN: Compt. rend. **128**, 26 (1899).
- (29) MOISSAN: Bull. soc. chim. (8) **19**, 947 (1898).
- (30) MOISSAN: Ann. chim. phys. (7) **18**, 289 (1899).
- (31) MOISSAN: Bull. soc. chim. (8) **21**, 904 (1899).
- (32) MOISSAN: Bull. soc. chim. (8) **31**, 549 (1904).
- (33) KRAUS: J. Am. Chem. Soc. **30**, 653 (1908).
- (34) ROOZEBOOM: Compt. rend. **110**, 134 (1890).
- (35) BENOIT: Bull. soc. chim. **33**, 908 (1923).
- (36) MENTREL: Compt. rend. **135**, 790 (1902).
- (37) ROEDERER: Compt. rend. **140**, 1252 (1905).
- (38) FRANKLIN AND KRAUS: Am. Chem. J. **20**, 850 (1898).
- (39) CADY: J. Phys. Chem. **1**, 707 (1896-7).
- (40) KRAUS: J. Am. Chem. Soc. **30**, 1323 (1908).
- (41) KRAUS: J. Am. Chem. Soc. **36**, 864 (1914).
- (42) KRAUS: Trans. Am. Electrochem. Soc. **21**, 119 (1912).
- (43) KRAUS: J. Am. Chem. Soc. **43**, 749 (1921).
- (44) GIBSON AND ARGO: Phys. Rev. **7**, 33 (1916).
- (45) GIBSON AND ARGO: J. Am. Chem. Soc. **40**, 1327 (1918).

- (46) GIBSON AND PHIPPS: J. Am. Chem. Soc. **48**, 312 (1926).
- (47) FRANKLIN AND KRAUS: Am. Chem. J. **23**, 277 (1900).
- (48) JOHNSON AND FERNELIUS: J. Chem. Education **7**, 981 (1930).
- (49) JAFFE: Ann. Physik. **32**, 148 (1910).
- (50) KRAUS AND LUCASSE: J. Am. Chem. Soc. **45**, 255 (1923).
- (51) CRAGOE AND HARPER: Bur. Standards, Sci. Papers, No. 369 (1920).
- (52) BRÖNSTED: Z. physik. Chem. **68**, 693 (1910).
- (53) KRAUS: Properties of Electrically Conducting Systems. American Chemical Society Monograph Series, Chemical Catalog Company, New York (1922).
- (54) BILTZ AND HUTTIG: Z. anorg. allgem. Chem. **114**, 241 (1920).

THE BEHAVIOR OF ELECTROLYTES IN DILUTE METHYL ALCOHOL SOLUTION

JOHN WARREN WILLIAMS

Laboratory of Physical Chemistry, University of Wisconsin, Madison

Received January 12, 1931

The development of the modern ideas concerning the nature of electrolytes in solution may be said to have begun with the theory of Arrhenius. But because the apparently anomalous behavior of strong electrolytes in solution could not be satisfactorily explained on the basis of the van't Hoff law of dilute solutions and the classical law of mass action it has been necessary to modify the original theory in a number of important details. More recent investigators have been able to overcome a number of these difficulties with the strong electrolytes by considering them to be completely ionized and assuming that the electrical forces between the ions account quantitatively for the observed deviations. The most successful of these interionic attraction theories we owe to Debye and Hückel who showed that it was possible to explain in a purely theoretical manner the empirical fact that the change of the activity coefficient of ions with concentration and the change of electrical conductance with concentration can be represented by a square root law, *provided* the solution is dilute enough so that the salt may be considered to be completely dissociated into ions. The explanation of these square root laws is based on the fact that the thickness of the ionic atmosphere surrounding each ion is proportional to the square root of the concentration. To obtain this result it is necessary to assume that the principal forces between the ions are given by Coulomb's law and are, therefore, inversely proportional to the square of the distances between them.

In this general discussion the properties of ions and molecules in non-aqueous solvents are considered. It is the purpose of

this paper to discuss the behavior of electrolytes in dilute methyl alcohol solution with particular reference to the interionic attraction theories of activity and conductance, and the extent to which they may be considered to be verified in this solvent. It will be well to have before us, then, the manner in which these theories are developed. Both theories involve two basic assumptions: (a) the electrolyte is completely dissociated; (b) the *principal* forces between the ions are Coulomb forces.

APPLICATION OF THE ACTIVITY THEORY TO THE AVAILABLE DATA FOR METHYL ALCOHOL SOLUTIONS

If the principal forces between the ions are Coulomb forces, that is, if they are inversely proportional to the square of the distance between them, a random distribution of the ions in the solution is impossible, but, owing to the electrical attractions, an ion of a given sign will, on the average, be surrounded by more ions of unlike sign than of like sign. The whole charge of this atmosphere of ions is, of course, equal and opposite to the charge of the ion responsible for it. But, due to the existence of the oppositely charged ionic atmosphere, there will be a difference between the electrostatic potential at the surface of the ion in an infinitely dilute solution where no atmosphere can form and the potential for a finite concentration where the atmosphere does form. The difference between these potentials, ψ , depends on the distance over which the total charge is spread out. There will also exist an average electrical density, ρ , at any distance, r , from the central ion. This density represents the excess of the electricity of opposite sign to be found, on the average, in a little element of volume which is imagined at the distance r from the ion in question, and is given by the ordinary Boltzmann principle, as follows:

$$\left(e^{-\frac{\epsilon\psi}{kT}} - e^{\frac{\epsilon\psi}{kT}} \right)$$

where ϵ is the charge of a positive univalent ion,

n is the number of positive ions in 1 cc.,

ψ is the average electrical potential due to ionic atmosphere,

k is the Boltzmann constant, and

T is the absolute temperature.

A uni-univalent salt in dilute solution is being considered here.

The next step in the development is an exceedingly important one. Debye and Hückel combined the equation of Poisson, which relates the electrical potential, ψ , and the density of electricity, ρ , with the equation of Boltzmann and eliminated ρ between them. But, as Kramers (1) and Fowler (2) have pointed out, such a procedure is legitimate when, and only when, certain fluctuation terms can be neglected as small. Thus, Debye and Hückel could use this method for very dilute solutions and in a solvent of sufficiently high dielectric constant. It is in this way that a result is obtained from which ψ may be determined.

$$\nabla^2 \psi = - \frac{4 \pi e n}{D} \left(e^{-\frac{e\psi}{kT}} - e^{\frac{e\psi}{kT}} \right)$$

$$= \frac{\pi e n}{D} \sinh \frac{e\psi}{kT}$$

For small potentials the quantity $\sinh \frac{e\psi}{kT}$ may be replaced by

$\frac{e\psi}{kT}$, the first term of the expansion of the exponential terms of the above equation, with the result,

$$\nabla^2 \psi = \frac{8 \pi n e \psi}{D k T} = k^2 \psi,$$

where

$$k^2 = \frac{8 \pi n e^2}{D k T}$$

Before proceeding with the solution of this equation, it may be pointed out that the mathematical simplicity of the original Debye and Hückel activity theory was based upon an approximation which made possible the combination of the Boltzmann and Poisson equations. Since this combination of equations involves a certain degree of approximation which is completely justified

only in very dilute solutions and in solvents of a sufficiently high dielectric constant it does not seem proper, as has been done in several cases (3), to attempt to extend the theory to the other solvents, to the more concentrated solutions, and to the water solutions containing ions of the higher valence types by considering not only the $\frac{e\psi}{kT}$ term but also the next members in the expansion of the hyperbolic sine function without considering the limitations which will have been imposed by the mathematical approximations made prior to the expansion.

It must be admitted that the magnitude of these fluctuation terms is at present unknown, nevertheless they may be proven to be small enough to be neglected only under the conditions mentioned above. There is undoubtedly a certain utility in making use of higher members of the expansion, but the treatment cannot be made exact in this manner alone.¹

Furthermore, as will be pointed out later, the effect of a close approach of ions and the effect of ions, particularly those of the higher valence types, in orienting the solvent dipoles in their immediate neighborhood has not been sufficiently considered in the attempted extensions of the theory to more concentrated solutions, to solvents of appreciably lower dielectric constant, and to cases where ions of the higher valence types are involved. These effects are capable of seriously limiting the correctness of the two basic assumptions which have been mentioned; in other words, the fundamental ideas of the Debye and Hückel theory itself do not lend themselves to an exact treatment in these cases.

To proceed briefly with the derivation, the average potential in the space around the selected ion depends only upon the distance, r , from it, therefore

$$\frac{d^2\psi}{dr^2} + \frac{2}{r} \cdot \frac{d\psi}{dr} = k^2 \psi.$$

¹This point is treated more exactly in forthcoming monographs by Falkenhagen (Hirzel, Leipzig) and by Williams and Falkenhagen (Chemical Catalog Co., New York).

The general solution of this differential equation is,

$$\psi = \frac{c}{r} e^{-kr} + \frac{c'}{r} e^{kr},$$

where c and c' are constants. The particular solution demanded by the boundary conditions is

$$\frac{c}{r} e^{-kr}$$

In words, the significant factor which represents the decrease in the density of electricity with the distance is the exponential e^{-kr} where k has the dimension of a reciprocal length and is given by the formula

$$\sqrt{\frac{4\pi}{DkT} \cdot \Sigma n_i z_i^2}$$

In this equation there has been introduced the necessary valence factor to make the expression conform to a salt of any valence type. The ionic strength is defined by the equation

$$\mu = \frac{1}{2} \Sigma n_i z_i^2$$

where n_i is the concentration of ion of i^{th} kind per 1000 grams of water, and z_i is the valence of ion of i^{th} kind in the solution.

The expression for the potential of an ion of the i^{th} sort is, by making a further approximation,

$$\psi = - \frac{e z_i k}{D} \cdot$$

From this potential ψ it is possible to determine the thermodynamic functions of the solution by methods which are beyond the scope of this article. Since we are concerned here with the expression for the activity coefficient of a salt in solution the end result will be written at once.

$$-\log f_s = \frac{\Sigma v_i z_i^2}{\Sigma v_i} \cdot \frac{1}{2} \frac{4\pi}{DkT} \sqrt{\frac{4\pi}{DkT} \cdot \Sigma n_i z_i^2}$$

where v_i is the number of ions formed by each molecule of the i^{th} kind.

We have made measurements of the activity coefficients of salts of valence types 1-1, 2-1, and 3-1 in very dilute methyl alcohol solution at 20°C. in order to test the validity of this equation in this solvent which has a dielectric constant of 30 at this temperature. The data for the salts of valence type 1-1 and 2-1 have been reported (4), but the data for the salt of valence type 3-1 are now to be reported for the first time. Substituting the values of the universal and variable constants, the equation for the logarithm of the activity coefficient of a salt in methyl alcohol solution becomes

$$-\log f_s = 2.0 z_1 z_2 \sqrt{\mu}$$

where z_1, z_2 are the valences of the ions in question, and μ is the ionic strength of the solution.

The activity coefficients were determined by means of the effect of solvent salts of different valence types on the solubility of highly insoluble saturating salts of the valence types mentioned. The apparatus for the saturation of the solutions was of the usual construction. The solvent and saturating salts were all subjected to a careful purification. The methyl alcohol was purified according to a recognized method.

The data for the solubility of the tri-univalent salt, luteo bromide, in methyl alcohol solutions of potassium sulfocyanate are given in table 1.

If one plots these, and the similar data of the previous article (4), by taking the logarithms of the solubility ratio, $\log \frac{s}{s_0}$, as abscissae against the square roots of the ionic strengths as ordinates, lines having but a slight curvature will be obtained. The limiting slopes of the resulting curves should be expressible by the factor $2.0 z_1 z_2$ of the above equation, if the Debye and Hückel activity theory in its simplest form is to be verified. The comparison of the limiting slopes obtained for the different systems with those predicted on the basis of this theory in its simplest form is made possible by table 2. An inspection of this table shows that for salts of the valence type 1-1 the limiting law

is obeyed with the same degree of exactness that one finds for similar systems in water solution. When the theory is tested in the case of saturating salts of valence type 2-1 it is found that the agreement with theory, though approximate, is less satisfactory,

TABLE 1

Solubility relations of tri-univalent salt luteo bromide in methyl alcohol at 20°C.
Solvent salt—KSCN

CONCENTRATION OF SOLVENT SALT <i>mols per liter</i>	NUMBER OF DETERMINATIONS	SOLUBILITY $\times 10^6$	μ	$\sqrt{\mu}$	$\log \frac{s}{\eta_0}$
0.000000	2	2.962	0.000178	0.01335	0.0000
0.000313	4	3.897	0.000546	0.02334	0.1193
0.000625	2	4.540	0.000898	0.02997	0.1855
0.001250	2	6.129	0.001618	0.04030	0.3158
0.002500	2	7.718	0.002963	0.05443	0.4159
0.005000	2	9.988	0.005599	0.07483	0.5279

TABLE 2

Comparison of experimental and theoretical slopes

SATURATING SALT	VA- LENCE TYPE	SOLVENT SALT	OB- SERVED LIMIT- ING SLOPE	THEO- RETICAL SLOPE
Croceo chloride.....	1-1	$C_6H_5SO_3Na$	1.7	2.0
Croceo chloride.....	1-1	KSCN	1.7	2.0
Croceo chloride.....	1-1	$Ba(ClO_4)_2$	2.5	2.0
Croceo chloride.....	1-1	$(C_6H_5COO)_2Sr$	1.7	2.0
Chloropentammine cobalt nitrate.....	2-1	KSCN	4.0	4.0
Chloropentammine cobalt nitrate.....	2-1	$Ba(ClO_4)_2$	8	4.0
Xantho chloride.....	2-1	KSCN	4.0	4.0
Xantho chloride.....	2-1	$Ba(ClO_4)_2$	6	4.0
Xantho chloride.....	2-1	$(C_6H_5COO)_2Sr$	6	4.0
Luteo bromide.....	3-1	KSCN	11.5	6.0

and in the case of the 3-1 valence type salt the conclusion is that there is no agreement. The data in this last case are admittedly meagre, but it may be pointed out that KSCN is undoubtedly the best solvent salt possible, and it was used in this particular case.

In order to make sure that these conclusions from the experi-

mental work were proper, Hansen and Williams (5) have studied the activity coefficients of ions of these three valence types in ethyl alcohol-water mixtures, in which it was possible to vary the dielectric constant of the solvent at will by changing the molecular ratio of ethyl alcohol to water. In all these experiments sodium chloride was used as solvent salt. The comparison of the observed and calculated limiting slopes of the curves which represent the experimental data are given in table 3.

TABLE 3
Experimental and theoretical slopes in ethyl alcohol-water mixtures at 25°C.

SOLVENT	DIELEC- TRIC CONSTANT	OBSERVED SLOPE	GALCU- LATED SLOPE
1. 1-1 Type salt. Croceo tetrannitrodiammincobaltiate			
1.00 mole fraction H ₂ O.....	78.8	0.50	0.50
0.80 mole fraction H ₂ O, 0.20 mole fraction EtOH.....	54.0	0.89	0.89
0.60 mole fraction H ₂ O, 0.40 mole fraction EtOH.....	41.4	1.31	1.32
0.40 mole fraction H ₂ O, 0.60 mole fraction EtOH.....	33.8	1.90	1.80
2. 1-2 Type salt. Croceo sulfate			
1.00 mole fraction H ₂ O.....	78.8	1.10	1.08
0.80 mole fraction H ₂ O, 0.20 mole fraction EtOH.....	54.0	1.74	1.76
0.60 mole fraction H ₂ O, 0.40 mole fraction EtOH.....	41.4	2.74	2.65
3. 3-1 Type salt. Luteo iodate			
1.00 mole fraction H ₂ O.....	78.8	1.52	1.51
0.80 mole fraction H ₂ O, 0.20 mole fraction EtOH.....	54.0	4.0	2.65
0.60 mole fraction H ₂ O, 0.40 mole fraction EtOH.....	41.4	6.1	3.98

This table definitely substantiates the statements made with regard to the behavior of these salts in methyl alcohol solution. One sees from it that in the case of the salt of simplest valence type, even when the dielectric constant of the medium has been reduced to 30, any deviation from the limiting slope is a second order effect; in the case of the 2-1 valence type salt the deviations are beginning to appear in the solvent of dielectric constant 40; and in the case of the 3-1 valence type salt the deviations are already large when the dielectric constant has been reduced to

two-thirds of the ordinary water value. Thus the figures found for the slopes in methyl alcohol of dielectric constant 30 could have been fitted into table 3 without difficulty.

There have been stated in the derivation of the activity equation the reasons why the writer does not believe that these discrepancies between theory and experiment can be explained by the expedient of including the higher terms in the expansion of the hyperbolic sine function. The step in the development of the theory leading to this hyperbolic sine function is not exact.

It has also been suggested that the fundamental assumptions upon which the theory is based will have to be modified if the theory is to account for the behavior of ions of higher valence types in solvents like methyl alcohol. Bjerrum (6) has indeed recognized the fact that the assumption which assigns to each and every ion an electrical effect must be modified. Bjerrum, in studying not only the stronger salt solutions in water but also the behavior of salts in methyl and ethyl alcohol solutions considers two ions within a certain critical distance from one another as "associated" and therefore without electrical effect. The number of these ion pairs has been calculated by the Boltzmann principle and the law of mass action. The activity theory is then applied to the remainder of the ions. This method, one which modifies the first basic assumption, actually gives a result for salt solutions of the higher valence types in water and for salt solutions of 1-1 valence type in methyl alcohol which more nearly approximates the result of experiment.

More recently there has been considerable discussion concerning the assumption of 100 per cent dissociation, and the consensus of opinion seems to be that the assumption is not justified, even in water solution. One certainly may expect even greater difficulties with it in methyl alcohol solution for the tendency of ions to associate, which is also increasing with increasing charge, will be more pronounced in this solvent. But quite apart from this difficulty the simple Debye-Hückel theory does not account for the influence of changes in the dielectric properties in the neighborhood of ions, particularly those of the higher valence types, for it is a well-known fact that the latter may exert a

segregating and orienting effect on dipole molecules, producing what is termed solvation. This effect will probably be of even greater significance in methyl alcohol than in water. The increased electric forces in the alcohol will cause larger deviations from the ideal laws of solutions, and the interionic attraction term which corrects for these deviations will become not only larger but much less amenable to reasonably exact calculation. It appears that the dielectric constant of the medium has been introduced into the equations in an inexact way, for from the statements made above it will be evident that the main part of D arises from the orientation of the solvent dipoles by the field of the ions and would therefore be different from the dielectric constant of an infinitely dilute solution, that is, the bulk constant for the pure solvent, which is actually used. A rigorous and quantitative examination of this particular point will no doubt involve considerable mathematical difficulty. It is an effect which would be expected to be of considerable importance in the case of ions of the higher valence types in methyl alcohol, and will make the application of Coulomb's law an approximation at best.

The validity of the use of Coulomb's law to express the principal forces between ions is directly involved in the close approach of two ions, particularly those of higher charge. In this event the force between them will be modified so as to involve some other power of the distance, and therefore the limiting or square root law, based upon the assumption of ordinary Coulomb forces always acting between ions, will be only approximate. Since the greatest discrepancies between experiment and theory are always noted in the cases of ions of the higher valence types it appears reasonable that this difficulty exists.

The corrections to be applied to Coulomb's law for the effect due to the close approach of ions and for the effect of segregation and orientation of solvent dipoles is an extremely difficult problem, yet it may eventually be solved.

Conclusion

From the standpoint of the interionic attraction theory of activity methyl alcohol may be considered to be a "water-like"

solvent. Differences in the behavior of electrolytes dissolved in it rather than in water are shown to be differences of degree only. The activity data in this solvent are valuable in that they demonstrate the inadequacy in non-aqueous solutions of the two basic assumptions of Debye and Hückel. They also indicate the nature of the additional assumptions which will have to be made if experiment and theory are to agree.

APPLICATION OF THE CONDUCTANCE THEORY TO THE AVAILABLE DATA FOR METHYL ALCOHOL SOLUTIONS

The interionic attraction theory of conductance has been developed by the use of methods which are similar to those used in dealing with the activity problem. The original theory of Debye and Hückel (7) was modified by Onsager (8), and it is in this latter form that it has been most successful. It cannot be said to have achieved the success that the activity theory has, yet it has stimulated a widespread interest in the accumulation of precision data for the conductances of salts in very dilute solution and in a number of solvents, and it has revolutionized the mode of treatment of these data. The reason that the conductance theory is less exact than the activity theory is not difficult to explain; in the conductance theory an irreversible process is being considered, while in the activity theory there is investigated an equilibrium condition to which the exact laws of thermodynamics may be applied. It means that a greater number of assumptions will be required in the former case, and an increase in the number of assumptions is always accompanied by a decrease in the accuracy with which a given phenomenon may be described.

In the classical theory of Arrhenius the decrease in equivalent conductance or molar conductance with increasing concentration was explained by assuming that the number of ions, that is, the number of carriers of the current, was continually decreasing. But in the interionic attraction theories the number of ions present is assumed to be always exactly proportional to the total concentration, so that the classical explanation for the decrease in molar conductance with increase in concentration must be discarded. In its place Debye and Hückel assumed that the con-

ductance changed with concentration because the mobilities of the ions changed, and it could be shown that the ionic atmosphere surrounding each ion provided the necessary explanation, because in addition to having a definite thickness, the atmosphere is characterized by a definite time of relaxation. As the ion moves through the liquid the atmosphere of opposite sign is continually forming about it and relaxing behind it. Since this process requires a definite period of time the ion will be moving away from an atmosphere of opposite sign, and will consequently be subjected to a force which decreases its mobility. This force is called the electrical force of relaxation. In addition there is a retarding force due to the fact that since the ions of opposite charge are moving in opposite directions through the solvent, and in moving tend to carry with them a certain amount of the solvent, each ion may be considered to be moving not in a stationary medium, but in one that is moving in a direction opposite to its motion. This is the so-called electrophoretic effect.

In the quantitative theory the magnitude of these forces has been calculated, both by Debye and Hückel and by Onsager. These calculations will not be given in this article; rather their result will be given in the simplified form adopted by Williams and Falkenhagen (9) in a recent review article devoted to the problem of conductance. The molar conductance at any concentration, Λ , is given by the expression

$$\Lambda = \Lambda_\infty - \Lambda_I - \Lambda_{II}$$

where Λ_∞ is the ordinary molar conductance at infinite dilution,

Λ_I is the electrical force of relaxation expressed as conductance,

Λ_{II} is the electrophoretic force expressed as conductance. Further,

$$\Lambda_I = \frac{1}{3} \frac{\epsilon_1 \epsilon_2}{DkT} \kappa \Lambda_\infty \frac{g}{1 + \sqrt{q}}$$

and

$$\Lambda_{II} = \frac{n_1 \epsilon_1^2 + n_2 \epsilon_2^2}{6 \pi \eta} \frac{1000}{\gamma} \frac{1}{9 \times 10^{11}} \kappa$$

In these equations

$$\kappa^2 = \frac{4 \pi}{DkT} \cdot \frac{\epsilon^2 N}{1000} \gamma \sum v_i z_i^2$$

$$q = \frac{L_1 z_2 + L_2 z_1}{(z_1 + z_2) (L_1 z_2^2 + L_2 z_1^2)} \cdot \frac{z_1 z_2}{z_1^2 z_2}$$

γ = the concentration of salt expressed in moles per liter.

N = the Avogadro number.

η = the coefficient of viscosity of the medium.

L_1, L_2 = the mobilities of individual ions, and the other symbols have the significance assigned to them in the discussion of the activity theory.

Introducing the universal constants, the expression for the molecular conductance of a simple electrolyte takes the form

$$\begin{aligned} \Lambda &= \Lambda_\infty - \left(\frac{0.985 \times 10^6}{(DT)^{\frac{1}{2}}} w \Lambda_\infty \sqrt{\sum v_i z_i^2} + \frac{29.0}{(DT)^{\frac{1}{2}} \eta} (\sqrt{\sum v_i z_i^2})^2 \right) \sqrt{\gamma} \\ &= \Lambda_\infty - \alpha \sqrt{\gamma} \end{aligned}$$

where

$$w = \frac{2 q}{1 + \sqrt{q}}$$

The molar conductance, Λ , is therefore given by an equation which is identical in form with the equation found empirically by Kohlrausch. It may then be said to be proven that the interionic attraction theory is adequate to explain Kohlrausch's law.

Since the conductance data now existent for dilute solutions in methyl alcohol are expressed in equivalent conductance rather than in molar conductance the above expression may be written for our discussion in terms of equivalent conductances, as follows:

$$\begin{aligned} \Lambda^* &= \Lambda_\infty^* - \left(\frac{0.985 \times 10^6}{(DT)^{\frac{1}{2}}} w \Lambda_\infty^* + \frac{29.0 (z_1 + z_2)}{(DT)^{\frac{1}{2}} \eta} \right) \sqrt{(z_1 + z_2) \gamma^*} \\ &= \Lambda_\infty^* - \alpha^* \sqrt{(z_1 + z_2) \gamma^*} \end{aligned}$$

where γ^* is the equivalent concentration,
 Λ^* and Λ_∞^* are equivalent conductances, and

$$\alpha^* = \frac{0.985 \times 10^8}{(DT)^{\frac{1}{2}}} w \Lambda_\infty^* + \frac{29.0 (z_1 + z_2)}{(DT)^{\frac{1}{2}} \eta}.$$

If the dielectric constant of methyl alcohol at 25°C. is assumed to be 30 and the values of the other constants are introduced, the limiting slope of the curve obtained when the equivalent conductance Λ^* is plotted against $\sqrt{\gamma^*}$ is given by the equation

$$\alpha^* = 109 + 1.15 w \Lambda_\infty^*$$

provided the discussion is limited to uni-univalent electrolytes. Fraser and Hartley (10) have determined the equivalent conductances of a number of salts of this valence type in methyl alcohol over the range of concentration, $\gamma^* = 0.0001$ to $\gamma^* = 0.002$ and have shown that the square root law is the correct one to explain the change of conductance with concentration. In order to test the interionic attraction theory further it is necessary to compare the limiting slopes calculated by it, using the relation given above, with the results of the experimental work. This comparison is given in table 4. In a number of cases the differences between the theoretical and actual slopes must be considered to be real deviations from the limiting law. It is true, however, that we cannot be absolutely sure with the data now available that the experiments have been made in sufficiently dilute solution.

More recently there has appeared an article by Unmack, Murray-Rust and Hartley (11) in which the conductances of a number of thiocyanates in very dilute methyl alcohol solution have been measured. The results for the thiocyanates of Li, Na, K, Rb, Cs and NH₄, that is, uni-univalent salts, are shown to be in excellent agreement with the Onsager modification of the Debye-Hückel limiting law. In the case of the thiocyanates of Ca, Sr, Ba, and Mg, that is, bi-univalent salts, the limiting slope determined by experiment is always much greater than that calculated using the theory. Indeed, this result might have been predicted from experience with conductance data for water

solutions, for in the case of the latter, the uni-univalent and bi-univalent salts obey the limiting law quite well, but when the data for bi-bivalent salts are considered the experimentally determined slopes are always much too high. It can be said with confidence that the chief difficulty rests not with the experimental determination of the mobilities which are required for the

TABLE 4

Conductance data for electrolytes of type 1-1 in methyl alcohol

$$\alpha_{\text{theor.}}^* = 1.15 w \Delta_m^* + 109$$

$$\alpha_{\text{theor.}} = (1.15 w \Delta_m^* + 109) \sqrt{2}, \text{ where } w = 0.5858$$

ELECTROLYTE	Δ_m^*	$\alpha_{\text{exp.}}$	$\alpha_{\text{theor.}}$	$\alpha_{\text{exp.}} - \alpha_{\text{theor.}}$
LiCl.....	90.9	223.5	240.8	-17.3
NaCl.....	97.0	230.5	246.6	-16.1
KCl.....	105.1	261.6	254.3	7.3
RbCl.....	108.7	281.5	257.7	23.8
CsCl.....	113.6	282.8	262.4	20.4
KF.....	94.0	236.1	243.7	-7.6
KBr.....	109.4	261.6	258.4	3.2
KI.....	114.9	260.2	263.6	-3.4
NH ₄ Cl.....	111.0	263.8	259.9	3.9
HCl.....	193.5	367.7	338.5	29.2
LiNO ₃	100.3	250.3	249.7	0.6
NaNO ₃	106.5	288.5	255.6	32.9
KNO ₃	114.6	345.0	263.3	81.7
RbNO ₃	118.2	355.0	266.8	88.2
CsNO ₃	123.0	378.9	271.3	107.6
AgNO ₃	113.0	451.1	261.8	189.3
NaBr.....	101.5	240.4	250.9	-10.5
NaOCH ₃	98.4	222.0	247.9	-25.9
NaClO ₄	115.1	280.0	263.8	16.2

calculation of α^* , but with the fact that most electrolytes are "associated" in methyl alcohol solution, even at great dilution.

If associated ions are present the basic assumptions of the interionic attraction theories, mentioned in the introduction, must be modified in any exact treatment. Deviations from Coulomb's law will undoubtedly occur when the distance between two ions becomes small, and they will be especially great in the case of polyvalent ions. It is only assumed that the *principal*

forces between the ions are given by Coulomb's law, and the theory does account for the influence of Coulomb's forces upon the mobilities of the ions. But to make the theory exact it is clear that the deviations must also be accounted for, and it will be necessary to determine the manner in which the forces vary as the distances of approach of the ions become smaller. This is definitely a problem of the newer physics.

One may also object to explaining the change of conductance with concentration as due solely to a change in the mobilities of the ions. With "ion pairs" present the conductance should decrease because the number of carriers is decreased as well, and a quantitative theory of conductance will therefore require that a method to measure the "degree of association" be made available. For this purpose the method used by Bjerrum, already referred to, has much to recommend it.

In this discussion no cognizance has yet been taken of the fact that a portion of the discrepancy between theory and experiment may be accounted for by the consideration of second and higher order terms, involving the dimensions of the ions. If the effect of these terms is included an improvement should result, yet it is our belief that this correction will not account for the major part of the deviations.

Conclusion

Methyl alcohol may also be considered to be a "water-like" solvent from the standpoint of the interionic attraction theory of conductance. Deviations between theory and experiment are sometimes marked even in the case of the simplest type of electrolyte in dilute solution, but these may be expected when the discrepancies which are found in water solution are considered. They demonstrate the same inadequacies in the basic assumptions underlying the conductance theory which were found in the case of the activity theory, and which are to be accounted for chiefly by *association* and *solvation* of the ions. The effect of both is to limit the applicability of Coulomb's law to express the interionic forces. The association of ions, prevalent in methyl alcohol solution, also makes it impossible to

completely account for the change in conductance with concentration by assuming that only changes in the ionic mobilities need be considered.

REFERENCES

- (1) KRAMERS: Proc. Acad. Sci. Amsterdam **30**, 145 (1927).
- (2) FOWLER: Trans. Faraday Soc. **23**, 434 (1927).
- (3) GRONWALL, LAMER AND SANDVED: Physik. Z. **29**, 358 (1928).
MÜLLER: Physik. Z. **29**, 78 (1928).
- (4) WILLIAMS: J. Am. Chem. Soc. **51**, 1112 (1929).
- (5) HANSEN AND WILLIAMS: J. Am. Chem. Soc. **52**, 2759 (1930).
- (6) BJERRUM: Kgl. Danske Videnskab. Selskab. Math. fys. Medd. **7**, 9 (1926).
- (7) DEBYE AND HÜCKEL: Physik. Z. **24**, 305 (1923).
- (8) ONSAGER: Physik. Z. **27**, 388 (1926); **28**, 277 (1927).
- (9) WILLIAMS AND FALKENHAGEN: Chem. Reviews **6**, 317 (1929).
- (10) FRASER AND HARTLEY: Proc. Roy. Soc. (London) A**109**, 351 (1925).
- (11) UNMACK, MURRAY-RUST AND HARTLEY: Proc. Roy. Soc. (London) A**127**, 228 (1930).

EQUILIBRIA IN NON-ELECTROLYTE SOLUTIONS IN RELATION TO THE VAPOR PRESSURES AND DENSITIES OF THE COMPONENTS

GEORGE SCATCHARD

*Research Laboratory of Physical Chemistry, Massachusetts Institute of Technology,
Cambridge*

Received January 31, 1931

The method to be outlined is an outgrowth of the treatment of gaseous systems used in the laboratory of the Massachusetts Institute of Technology. It may be regarded also as a quantitative development of the treatment of Hildebrand, although it disagrees with his ideas in some important details, or as a method of freeing the van Laar treatment from the inadequacies of the van der Waals equation. In this paper is presented the simplest first approximation. Though its success is not astounding, it is hoped that the presentation will show some of the reasons for the limitations of so simple a theory, as well as indicate some of the possibilities of improvement. It will also serve as a comparative review of the different theories.

The general method is to make all variations of composition¹ and temperature at such low pressures that the perfect gas laws are applicable, and to make the pressure variations at constant temperature and composition (1). There is no obvious method of extending to liquids the treatment of free energy itself, but progress may be made through the energy. We may express the energy of a mole of liquid as $U_1 = U_0 - u$. U_0 is the molal energy of the gas at zero pressure, and we need not consider it further for it is a function only of the temperature, independent of the pressure, the state of aggregation, and of the composition unless there is a chemical change which persists at zero pressure.

¹ "Composition" is used in the stoichiometric sense of ratio of the components, disregarding any chemical reaction which may occur.

We shall call u the cohesive energy. At sufficiently low vapor pressures it is equal to $L - RT$. For those substances for which the computation can be made, that is, those for which the constants of the Beattie-Bridgeman equation of state (2) have been determined, u is about 1 per cent greater than $L - RT$ at the boiling point, the difference varying from 0.8 per cent for ammonia to 1.3 per cent for argon. So, at the boiling point or below, we may take $L - RT$ as the cohesive energy.

For most substances the heat of evaporation has not been determined, but we may make use of an extension of Hildebrand's amendment (3) of Trouton's law. We may express the vapor pressure as

$$\log \frac{p}{T} = \frac{B - A/T}{1 + 0.05(B - A/T)} \quad L - RT = \frac{2.303 RA}{[1 + 0.05(B - A/T)]^2}$$

By the Trouton-Hildebrand law, B has the same value for all normal liquids, and the single specific constant, A , may be determined from a single vapor pressure or boiling point measurement. Taking B as 4.7 for normal liquids, as 5.0 for esters, aldehydes and ketones, and as 5.7 for water and the alcohols, the agreement is excellent for pressures from 3 to 2000 mm. The few available measurements indicate that the agreement is not so good for lower pressures. Since the measurements of boiling points are generally more accurate than those of vapor pressure, we shall use them to measure A and u . We shall be interested in the cohesive energy per unit volume or cohesive energy density, u/V . Since the measurements of volume are generally made at 20°, we shall use u and u/V calculated for that temperature.

Our first problem is to determine the shape of the energy composition curve for liquid mixtures. We shall attack it by considering an ideal solution—one with zero change in volume and energy on mixing. The forces acting on a given molecule must be independent of the composition, but what is the relation between the forces acting on a molecule of the first species to those acting on one of the second? We might expect that two substances should form an ideal solution, not when their cohesive

energies per mole are equal, but when they have the same energy density. This corresponds to the facts.

For non-ideal solutions also we shall neglect the change in volume, and we shall consider only the case in which the interaction between any pair of molecules is independent of the composition. We may then split the cohesive energy of a mole of the mixture in the following way

$$u_s = \frac{a_{11}V_1^2x_1^2 + 2a_{12}V_1V_2x_1x_2 + a_{22}V_2^2x_2^2}{V_1x_1 + V_2x_2}$$

where the a 's are constants. For the pure components $u_1 = a_{11}V_1$ and $u_2 = a_{22}V_2$, so that a_{11} and a_{22} are the cohesive energy densities of the components. The energy increase of mixing is

$$\begin{aligned}\Delta U &= u_1x_1 + u_2x_2 - u_s = (a_{11} - 2a_{12} + a_{22})V_1V_2 \frac{x_1x_2}{V_1x_1 + V_2x_2} \\ &= A_{12}V_1V_2 \frac{x_1x_2}{V_1x_1 + V_2x_2}\end{aligned}$$

From our assumption of additivity of volumes, it follows immediately that $\Delta H = \Delta U$. The next step is more daring, although it or its equivalent is made in every physical theory of liquid solutions, and some of the justifications are more entertaining than convincing. We shall satisfy ourselves with Hildebrand's statement (4) that when "orienting and chemical effects are absent and the distributions and orientations are random," the entropy of mixing is the same as for an ideal solution. Then

$$\Delta F = \Delta U + x_1RT \ln x_1 + x_2RT \ln x_2$$

and

$$\mu_1 - \mu_{10} = RT \ln a_1 = RT \ln x_1 + A_{12}V_1V_2 \left(\frac{x_2}{V_1x_1 + V_2x_2} \right)^2$$

The expressions are simpler using volumes as units rather than moles, when the energy increase per unit volume of the mixture is A_{12} multiplied by the product of the volume fractions of the components, and the chemical potential per unit volume of one

component is equal to that in an ideal solution plus A_{12} multiplied by the square of the volume fraction of the other component. The application to vapor pressures, solubility of solids, and miscibility of liquids follows by the standard thermodynamic methods.

To determine the behavior of a mixture from the properties of its components, it is necessary to make one more assumption, regarding the value of a_{12} . We will assume that the cohesive energy behaves as the gravitational energy between point masses, or the electrostatic energy between point charges, and that

$$a_{12} = \sqrt{a_{11}a_{22}}.$$

Then

$$A_{12} = (\sqrt{a_{11}} - \sqrt{a_{22}})^2,$$

which is twice the difference between the arithmetic and the geometric mean of a_{11} and a_{22} . The cohesive energy densities must differ by 20 per cent of their mean, about the difference for benzene and carbon bisulfide, if A_{12} is to be 1 per cent of the mean. The accuracy of our assumption must then be very great if we are to predict the behavior of mixtures to a good approximation.

This assumption implies homogeneous molecules. Given a pair like naphthalene and acetone, which have nearly the same energy densities while one is homogeneous and the other has a much stronger field in one part than another, we should not expect them to give ideal solutions. They should be described more accurately by a method analogous to that of Langmuir.

Hildebrand (5) uses the "internal pressure," $\left(\frac{\partial U}{\partial V}\right)_T$ as a criterion instead of $\frac{u}{v}$, although he suggests the latter as an approximate measure of the former. For a van der Waals fluid, the two are identical. Our derivation indicates that, when they differ, it is the latter which is important rather than the former.

Hildebrand (6) and Mortimer (7) also state that the deviations

should be at least approximately proportional to the difference between the internal pressures, which is always larger than the square of the difference between their square roots, and is very much larger when the difference is small, so that nearly ideal solutions should be much rarer by the Hildebrand-Mortimer hypothesis.

Heitler (8), by assuming that the liquid mixture corresponds to a cubic crystal lattice, obtains an expression similar to ours, except that mole fractions replace volume fractions. His basic assumption is equivalent to assuming equal molal volumes of the components, and his results should be limited to this case, for which his equation is the same as ours.

Langmuir's equation (9) may be put in the same form as ours by replacing energy density by energy per unit surface, and volume fraction by surface fraction. It involves the assumption that the surface of contact between two surfaces is proportional to the product of the surfaces, which seems to me very doubtful; Langmuir makes the further assumption that the surface of any molecule is proportional to the two-thirds power of its volume, which seems to me certainly wrong.

van Laar's equation (10) is the same as ours except that van der Waals' b_1 replaces V_1 , and van der Waals' a_1/b_1 replaces a_{11} .

Dolezalek's theory (11) may be expressed as the assumption that $a_{12} = (a_1 + a_2)/2$ unless there is chemical action. In this case it makes no difference whether the composition be expressed as mole, surface, or volume fraction. The long polemic between van Laar and Dolezalek and their adherents is then based on the small difference between the arithmetic and geometric means. Dolezalek is in the peculiar position of denying that a force is physical unless it behaves as no known physical force behaves. Of course there are more pragmatic grounds for not accepting Dolezalek's theory as general (12).

We have to test, then, four consequences of the theory: the shape of the curve for the change of heat content or free energy on mixing; the equality of these two functions; the equality of each to the value calculated from the cohesive energy densities; and the use of the extended Trouton-Hildebrand law to compute

these energy densities. In general the last two cannot be tested independently.

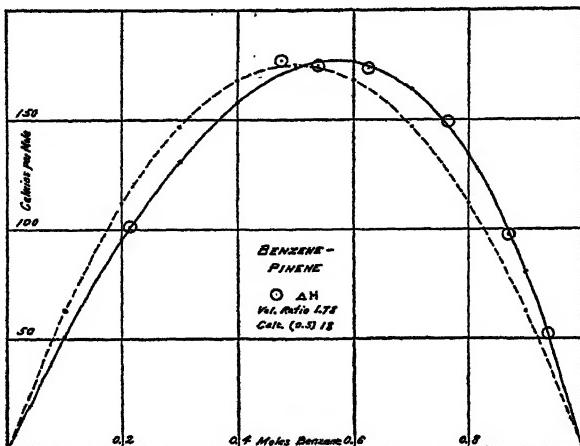


FIG. 1. HEAT OF MIXING AND VOLUME
BENZENE-PINENE

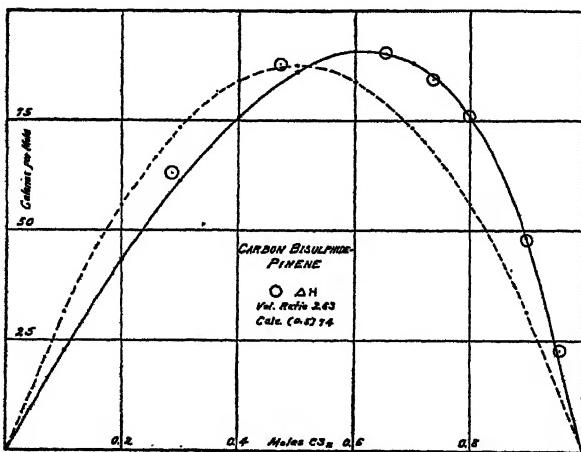


FIG. 2. HEAT OF MIXING AND VOLUME
CARBON BISULFIDE-PINENE

The shape of the curve is shown better by the measurements of heat content (14), because they appear to be more reliable than those of free energy, and because there happen to be some for

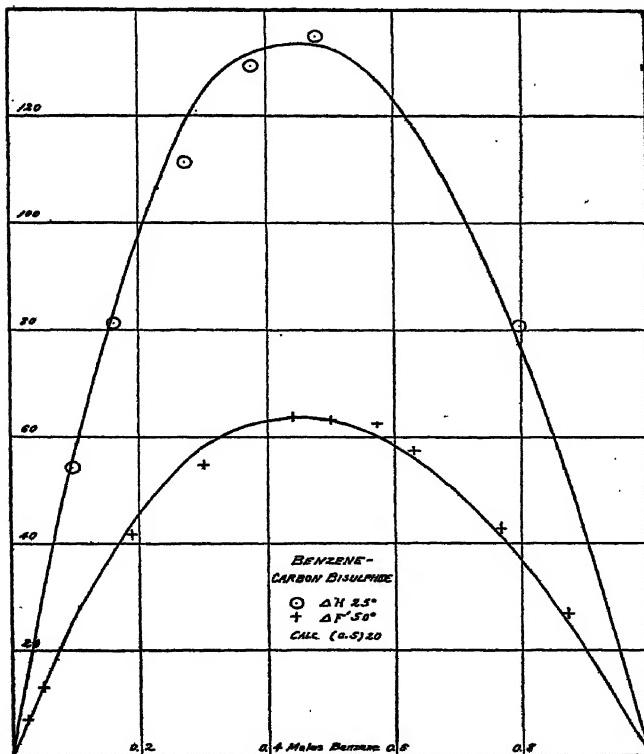


FIG. 3. HEAT AND FREE ENERGY OF MIXING
 BENZENE-CARBON BISULFIDE
 $\Delta F' = \Delta F - x_1 RT \ln x_1 - x_2 RT \ln x_2$

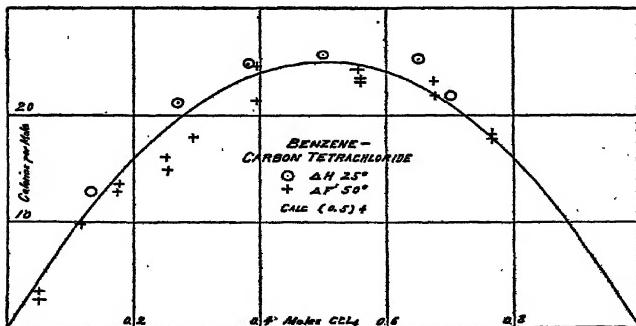


FIG. 4. HEAT AND FREE ENERGY OF MIXING
 BENZENE-CARBON TETRACHLORIDE

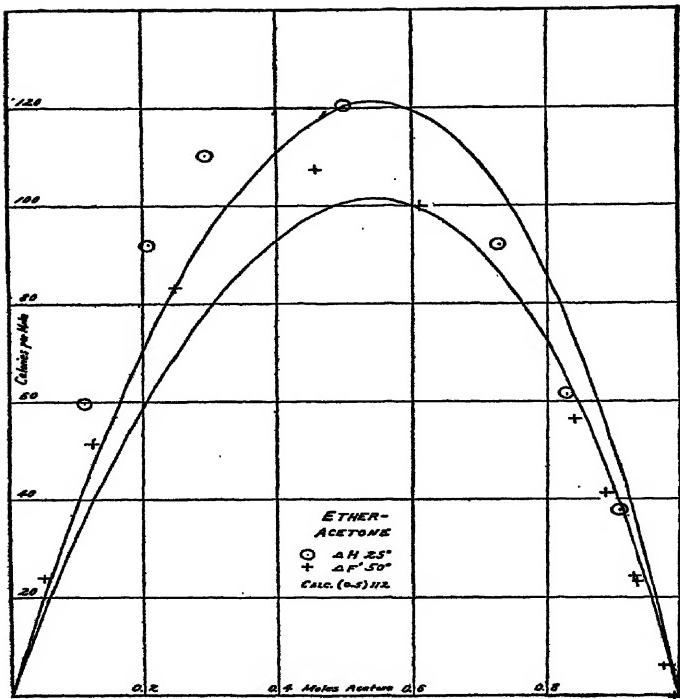


FIG. 5. HEAT AND FREE ENERGY OF MIXING
ETHER-ACETONE

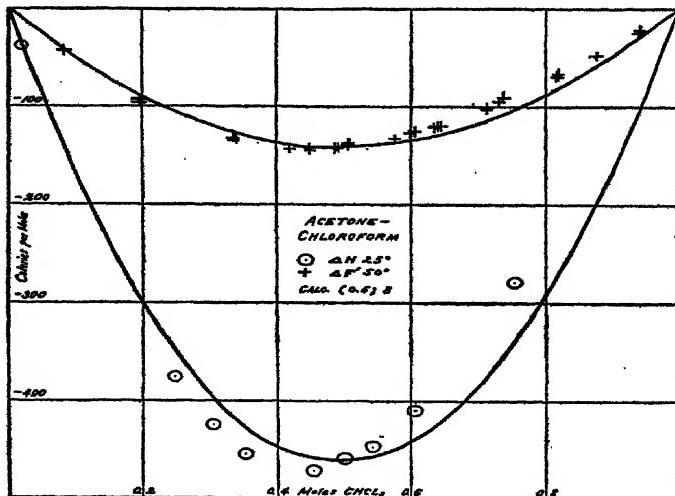


FIG. 6. HEAT AND FREE ENERGY OF MIXING

substances which would be expected to behave normally and which have widely different volumes. The evidence in figures 1 and 2 is clearly in favor of the volume fraction rather than the mole fraction. The calculated value is good in one case, and poor in the other.

Figures 3 to 6 show ΔH and $\Delta F' = \Delta F - x_1 RT \ln x_1 - x_2 RT \ln x_2$ for all the systems for which Hirobe (14) found large heats of mixing and for which the change in free energy can be computed

TABLE I
Solubility of naphthalene at 20°

SOLVENT	u/V	V	SOLUBILITY		$\log a/z$	
			Measured	Calculated	Measured	Calculated minus measured
<i>calories per cc.</i>						
Naphthalene (ideal).....	101.5	123	0.261	0.261	0.000	
Chlorobenzene.....	93.4	101.63	0.256	0.256	0.008	0.000
Benzene.....	83.9	88.89	0.241	0.240	0.034	0.002
Toluene.....	81.0	106.31	0.224	0.228	0.064	-0.006
Carbon tetrachloride.....	76.1	96.45	0.205	0.210	0.104	-0.010
Hexane.....	54.2	130.47	0.090	0.067	0.462	0.128
Aniline.....	154.5	91.06	0.130	0.110	0.302	0.073
Nitrobenzene.....	143.1	101.95	0.243	0.158	0.080	0.187
Acetone.....	97.0	73.34	0.183	0.260	0.153	-0.152
n-Butyl alcohol.....	123.7	91.45	0.0495	0.232	0.721	-0.671
Methyl alcohol.....	213.7	40.44	0.0180	0.00075	1.161	1.380
Acetic acid.....	172.6	57.23	0.0456	0.0540	0.767	-0.083

(from vapor pressure measurements) (15). For benzene-carbon tetrachloride and ether-acetone ΔH and $\Delta F'$ are nearly equal. The calculated value agrees well in the first case, but less well in the second. For benzene-carbon bisulfide and chloroform-acetone, the discrepancy is large. In each case $\Delta F'$ is nearer to the calculated value than it is to ΔH .

Lest these results give the impression that the usefulness of the theory is extremely limited, let us look in table 1 at the measured and computed solubilities of naphthalene at 20°. The measured values are interpolated from the measurements of Ward (16).

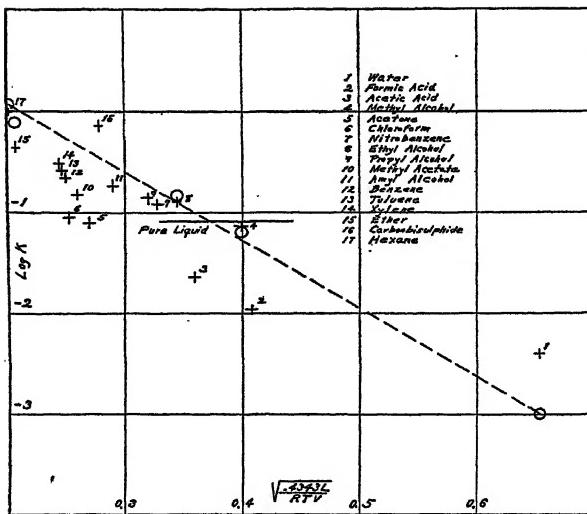


FIG. 7. KETO-ENOL ISOMERISM IN ACETOACETIC ESTER
VERY DILUTE SOLUTIONS

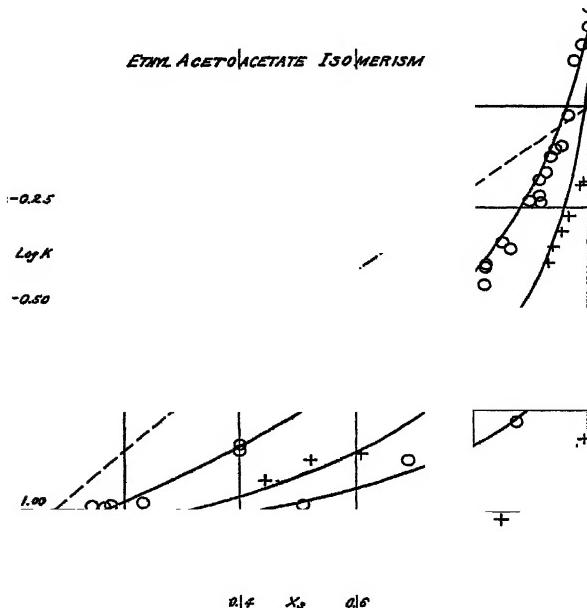


FIG. 8. KETO-ENOL ISOMERISM IN ACETOACETIC ESTER
EFFECT OF CONCENTRATION

Solvents (in order of decreasing intercepts): hexane, carbon bisulfide, benzene, ethyl alcohol.

The cohesive energy density of naphthalene itself is computed from the heats of fusion and sublimation; those of the solvents are computed by the equations given. For the non-polar solvents the agreement is as good as could be expected, and for aniline it is not much worse. For the more polar solvents the discrepancies are large, except for acetic acid, where the agreement is probably a coincidence.

In our treatment of solutions we are inclined to forget that for a chemist the most important equilibria are chemical. In the simplest case, isomerism, only two molecular species are involved and the volumes may be taken as equal. The equations for three component mixtures will not be presented. At infinite dilution, $\log K$ should be a linear function of the square root of the energy density of the solvent. The intercept on the zero axis is $\log K$ for the perfect gas system, and the slope is proportional to the volume of the reactants and to the difference in the square roots of their energy densities. From these quantities and the reaction constant for the solvent-free system, it is possible to calculate the constant for a solution of any composition. If the energy densities of the reactants are known, one measurement of the equilibrium constant is sufficient to determine all.

The only system which has been measured in a large variety of solvents is acetoacetic ester. Both forms should be highly polar, as are many of the solvents. The theory is doubtless insufficiently developed to fit this case. In figure 7 the circles represent the measurements of Hantsch (17) on very dilute solutions, and fall on a straight line better than would be expected. The crosses represent the measurements of Kurt Meyer, generally on 3 per cent solutions, and the discrepancies are large. It might be noted that the Hildebrand-Mortimer law would lead to $\log K$ at infinite dilution independent of the solvent.

Figure 8 shows the change of constant with composition for four solvents. The broken line is that calculated for hexane, and the agreement is very poor. The full lines are calculated on the more general theory which permits a_{12} to be independent of a_{11} and a_{22} . To compute these curves we need the value of the constant for no solvent, one point on each curve, and one other con-

stant, which is the same for all the curves. The reason why they appear not to belong to the same family is that they are plotted against the mole fraction rather than the volume fraction. Here the agreement is excellent. The only difficulty is that the values of the constants require that the vapor pressure of the enol form be more than a hundred thousand times that of the keto form. The experimental evidence indicates that the ratio is of the order of a hundred, and the larger value seems out of the question. Even the ratio of a hundred is quite surprising, for we might expect the enol form to have the smaller pressure. So this system, which appears simple on paper, may be much too severe a test of the theory. It is possible that the α factors vary with the composition, though more experimental information is necessary to establish such a variation, or it may be, as suggested by Knorr (18), that there is equilibrium between three forms instead of two.

SUMMARY

The properties of liquid mixtures are calculated from the following assumptions (of which only the first is general).

The energy of a liquid may be split into two parts: the energy under perfect gas conditions, which is additive and may be ignored; and a second part which is nearly equal to the energy of liquefaction, and which may be calculated approximately from the boiling point.

The energy of mixing of two liquids depends upon their difference in cohesive energy density.

The interaction between two molecules is independent of the composition.

The interaction between unit volumes of two different species is the geometric mean of those for the two species taken separately.

The entropy of mixing is the same as for perfect gas conditions.

The theory is compared with other theories of liquid solutions, and with some measurements of the heat of mixing, of equilibria with the gaseous phase and with one solid component, and of the chemical equilibrium between isomers.

BIBLIOGRAPHY

- (1) GILLESPIE: J. Am. Chem. Soc. **47**, 305 (1925); **48**, 28 (1926).
BEATTIE: Phys. Rev. **31**, 680 (1928).
- (2) BEATTIE AND BRIDGEMAN: Proc. Am. Acad. Arts Sci. **63**, 229 (1928); Z. Physik **62**, 95 (1930).
- (3) HILDEBRAND: J. Am. Chem. Soc. **37**, 970 (1915).
- (4) HILDEBRAND: ibid. **51**, 69 (1929).
- (5) HILDEBRAND: Solubility, pp. 69, 99. Chemical Catalog Co., New York (1924).
- (6) HILDEBRAND: J. Am. Chem. Soc. **51**, 79 (1929).
- (7) MORTIMER: ibid. **44**, 1416 (1922); **45**, 633 (1923).
- (8) HEITLER: Ann. Physik [4] **80**, 630 (1926).
- (9) LANGMUIR: Third Colloid Symposium Monograph, p. 3. Chemical Catalog Co., New York (1925).
- (10) VAN LAAR: Z. physik. Chem. **72**, 723 (1910); **83**, 599 (1913).
- (11) DOLEZALEK: ibid. **64**, 727 (1908).
- (12) HILDEBRAND: Solubility, p. 83. Chemical Catalog Co., New York (1924).
- (13) HIROBE: J. Faculty Sci. Imp. Univ. Tokyo **1**, 155 (1925).
- (14) SAMESHIMA: ibid. **1**, 63 (1925) for benzene-carbon bisulfide and ether-acetone.
ZAWIDSKI: Z. physik. Chem. **35**, 129 (1900) for benzene-carbon tetrachloride and acetone-chloroform.
- (15) WARD: J. Phys. Chem. **30**, 1316 (1926).
- (16) HANTSCH: Ber. **43**, 3049 (1910); **44**, 1771 (1911).
- (17) MEYER: Ann. **380**, 226 (1911); Ber. **47**, 826 (1914).
- (18) KNORR, ROTHE AND AVERBECK: Ber. **44**, 1138 (1911).

ELECTRODEPOSITION OF METALS FROM NON-AQUEOUS SOLVENTS

L. F. AUDRIETH AND H. W. NELSON

Department of Chemistry, University of Illinois, Urbana

Received January 12, 1931

INTRODUCTION

Electrochemists, particularly electroplaters, have paid comparatively little attention to the use of non-aqueous solvents. However, several researches in the past few years have indicated that developments will certainly include a study of the deposition of metals from solutions of their salts in solvents other than water. It may seem a bit premature to stress the application of non-aqueous solvents to the problem of electrodeposition of metals, particularly when the fundamental electrochemical reactions in aqueous solution are still so little understood and subject to so much dispute. The question may be asked whether it is of advantage to introduce another variable in the form of a new solvent when the effect of such factors as temperature, concentration of solute, current density, addition agents, etc., has been defined only in a qualitative way. However, our knowledge of electrodeposition from aqueous solution is limited to a comparatively small number of metals and non-metals, and attempts to discharge the remainder of the elements from aqueous solutions of their compounds, and under a variety of conditions, have thus far failed. Experience has shown that some metals, as such, will probably never be discharged from aqueous solution because of their reactivity with water. On the other hand, solvents are known which either do not attack these metals or which react with them only very slowly, and if such solvents give conducting solutions of compounds of these metals, their possible application as media for electrodeposition is worth investigation. De-

spite the comparatively small number of researches which have dealt with this subject, the success which has attended some of them should bring home to all electrochemists the applicability to their field of endeavor of a statement made by Conant (1) that, "Much important chemistry (particularly important to the organic chemist) has been obscured by our slavish devotion to water."

The experimental work thus far carried out brings to light several rather salient facts. A whole variety of solvents, *viz.*, sulfur dioxide, formamide, liquid ammonia, acetamide, pyridine, acetic acid, ethyl bromide, etc., differing widely in physical properties and chemical character, has been employed. Apparently no satisfactory basis for the choice of any one solvent has been given. It may safely be said that scientific curiosity has in most instances prompted these investigations, which have been for the greater part qualitative in nature. Even so, the results obtained have been rather surprising and quite encouraging.

Not only have many different solvents been employed, but the choice of a suitable electrolyte for solution in any specific medium has been hampered by a lack of information. Despite the immense amount of routine effort which has been expended in studying solubilities, we find that the data are limited largely to aqueous solutions. The literature contains few reliable references concerning solubilities in such solvents as pyridine, acetone, the various alcohols, acetonitrile, etc. In the case of liquid ammonia qualitative observations only are available.

Not only does the choice of the proper salt for any one solvent offer a serious obstacle, but the preparation of many anhydrous compounds, particularly the nitrates, bromides, iodides, thiocyanates and cyanides—these come nearest to being universal solutes—is fraught with difficulties. In some cases the presence of water, introduced as water of hydration, makes no difference so far as the final result is concerned, but in many instances the addition of water changes the solvent character in a rather surprising manner. Thus, water behaves as a weak acid in liquid ammonia, whereas it is basic towards acetic acid. In line with

the idea of using anhydrous salts, every effort should also be made to employ anhydrous solvents. The presence of a trace of water has been shown in some cases to prevent electrodeposition of certain metals, yet, on the other hand, to improve the character of the deposits obtained.

HISTORICAL

The first recorded instance where non-aqueous solvents were used for the electrodeposition of a metal involved the electrolysis of solutions of lithium chloride both in acetone (2) and in pyridine (3) as a means for preparing lithium metal. The same investigator, Laszynski, also succeeded in discharging potassium from a solution of the thiocyanate in pyridine and prepared the mercury amalgams of potassium, sodium and ammonium by electrolysis of soluble salts of these metals in acetone using a mercury cathode. Kahlenberg (4) continued the study of the deposition of lithium from solutions of lithium chloride in pyridine and obtained bright, shiny deposits. He subsequently extended his investigations to include other non-aqueous solvents such as aniline, benzonitrile, and quinoline in addition to acetone and pyridine (5). Bright, adherent deposits of silver were obtained from solutions of silver nitrate in all five solvents. Lead was deposited from lead nitrate in pyridine; antimony from a solution of antimony trichloride in methyl alcohol. Attempts to plate bismuth from solutions of bismuth trichloride in methyl alcohol yielded only black, spongy, non-adherent products.

DEPOSITION FROM PYRIDINE

The problem of electrodeposition from pyridine has in recent years been studied very carefully by Müller (6) and his coworkers in Graz. Müller took special pains to make his materials absolutely anhydrous and thus succeeded in discharging metals from solutions of their salts in pyridine where previous investigators had failed. The decomposition potentials of various metallic salts, as well as single potentials of the metals against solutions containing the respective ions in pyridine were determined.

Silver (6a, 6b), magnesium (6d), calcium (6d), zinc (6d), copper (6d), iron (6d), potassium (6c), sodium (6c), and lithium (6c) were discharged cathodically. No deposit of beryllium (6d) was obtained upon electrolysis of a solution of beryllium bromide in pyridine. Müller also investigated various other solvents (6c), but with less success. Neither lithium nor magnesium could be deposited from solutions of their respective nitrate and bromide

TABLE I
Electrodeposition from pyridine

METAL	SOLUTE	CURRENT DENSITY PER SQUARE CENTIMETER <i>amperes</i>	METAL BASE	REFERENCE
Li.....	LiCl	0.001-0.003	Pt or Fe	Laszynski
	LiNO ₃		Pt or Fe	Kahlenberg, Patten and Mott, Müller
Na.....	NaI, NaNO ₃ , NaSCN		Pt	Müller
K.....	KSCN		Pt	Müller
Ca.....	Ca(NO ₃) ₂	0.0007 at 18°	Pt	Müller
Ba.....	BaI ₂		Hg	Hevesy
Ag.....	AgNO ₃		Pt	Kahlenberg, Müller
Cu.....	Cu ₂ I ₂		Pt	Kahlenberg, Müller
Fe.....	FeCl ₃		Pt	Kahlenberg, Müller
Pb.....	Pb(NO ₃) ₂		Pt	Kahlenberg
Mg.....	MgBr ₂	0.0003	Pt	Müller
Zn.....	ZnI ₂		Pt	Müller
Mn.....	MnCl ₂	No deposits	Pt	Müller
Be.....	BeBr ₂	No deposits	Pt	Müller
U.....	UO ₂ Cl ₂	No deposits	Pt	Pierle
Ra.....	RaBr ₂	No deposits	Pt	Coehn

in acetonitrile. However, a deposit of silver from silver nitrate in this solvent was readily obtained. Calcium did not discharge from a solution of the nitrate in amyl alcohol.

Hevesy (7) found that the alkaline earth iodides were quite soluble in pyridine, but was unable to deposit these directly upon a platinum cathode. By using a mercury cathode he did succeed in obtaining a 30 per cent barium amalgam. Unsuccessful attempts have also been made to electrodeposit radium (8) and uranium (9) from solutions of their compounds in pyridine.

ACETONE AS AN ELECTROLYTIC SOLVENT

Acetone has also found some application in electrodeposition of metals. Deposits of strontium and sodium can be obtained from saturated solutions of the respective iodides when electrolyzed at high current densities. Electrolysis of solutions of the chlorides of cadmium, tin, antimony, bismuth, and arsenic at moderate current densities also yields the respective metals

TABLE 2
Electrodeposition from acetone

METAL	SOLUTE	CURRENT DENSITY PER SQUARE CENTIMETER	METAL BASE	REFERENCE
amperes				
Li.....	LiCl	High	Cu	Laszynski
	LiCl	0.001	Pt	Patten and Mott
Na.....	NaSCN		Hg	Laszynski
	NaI	0.2	Pt	Patten and Mott
K.....	KSCN		Hg	Laszynski
NH ₄ ⁺	NH ₄ SCN		Hg	Laszynski
Ca.....	CaCl ₂	0.001	Pt	Siemens
Sr.....	Sr(NO ₃) ₂	0.001	Pt	Siemens
	SrI ₂	0.2	Pt	Patten and Mott
Cu.....	CuCl ₂	0.1	Pt	Patten and Mott
Ag.....	AgNO ₃		Pt	Kahlenberg
Zn.....	ZnCl ₂		Pt	Patten and Mott
Cd.....	CdCl ₂	0.001	Pt	Patten and Mott
Fe.....	FeCl ₃	0.2	Pt	Patten and Mott
Sn.....	SnCl ₂	0.001	Pt	Patten and Mott
As.....	AsCl ₃	0.136	Pt	Patten and Mott
Sb.....	SbCl ₃	0.0007	Pt	Patten and Mott
Bi.....	BiCl ₃	0.028	Pt	Patten and Mott

(10). Patten and Mott found that zinc (11) and lithium (12) could be deposited from solutions of their chlorides in this solvent. Siemens (13) also studied the deposition of lithium, sodium, potassium and calcium from acetone in an effort to utilize such a process commercially.

FORMAMIDE AS A SOLVENT

On the basis of a study of the conductivity of various inorganic salts in formamide, Walden (14) suggested that this solvent

might be employed very advantageously for the electrodeposition of metals. A preliminary study was subsequently made by Röhler (15), who employed the following compounds as electrolytes for the deposition of the respective cations: $\text{Pb}(\text{NO}_3)_2$, PbCl_2 , CuSO_4 , Cu_2Cl_2 , ZnO , ZnCl_2 , SnCl_2 . Metallic anodes composed of the metals to be deposited were used. A quantitative study of the electrolytic reactions showed that the anodic losses were invariably found to be greater than the weight of metal deposited. These differences were ascribed to the solvoly-

TABLE 3
Electrodeposition of metals from formamide at 100°

COMPOUND	SOLUTE PER 15 CC. SOLVENT	CURRENT DENSITY PER SQUARE CENTIMETER	TYPE OF DEPOSIT
	grams	amperes	
$\text{Zn}(\text{CN})_2$	0.5	0.03	Adherent. Continued electrolysis yielded spongy material
$\text{Cd}(\text{CN})_2$	1.4	0.02	Smooth and adherent
$\text{Pb}(\text{NO}_3)_2$	3.0	{ 0.03 0.06	Non-adherent Crystalline. Deposit is not uniform, but adheres only in spots
SnI_4	1.0	0.04	Smooth and adherent
$(\text{NH}_4)_2\text{SnCl}_6$	2.0	0.04	Excellent deposition
$\text{Co}(\text{C}_2\text{H}_5\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$	2.0	{ 0.02 0.05	Poor Better deposition; adherent
$\text{Ni}(\text{C}_2\text{H}_5\text{O}_2)_2$	Saturated	0.018	Fair and adherent
$\text{Ni}(\text{NH}_4)_2\text{Br}_2$	2.0	0.025	Good and adherent

sis of the respective electrolytes with the resultant formation of the metallic formamide derivative and the free acid. Chemical action of the acid, so formed, upon the metallic anodes was postulated to account for these discrepancies. It was found that dissolved oxygen, as well as exposure of the solution to air, influenced the character of the deposit very decidedly. These observations have recently been checked and confirmed by the investigations of Yntema and Audrieth (16). In addition to the metals which Röhler was able to plate from formamide these investigators succeeded in depositing cadmium, nickel and co-

balt. Magnesium, aluminum, iron, and some of the more active metals could not be discharged.

ACETAMIDE AS A SOLVENT

Another solvent which resembles formamide closely is acetamide. Although a solid at ordinary temperature, it is liquid at 100°C., and has been found to be an excellent solvent for a great many inorganic salts. The compounds $Zn(CN)_2$, $Cd(CN)_2$,

TABLE 4
Electrodeposition of metals from acetamide at 100°

COMPOUND	SOLUTE PER 10 GRAMS OF SOLVENT	CURRENT DENSITY PER SQUARE CENTIMETER	TYPE OF DEPOSIT
$Zn(CN)_2$	grams 0.177	amperes 0.05	Deposit localized
$Cd(CN)_2$	0.164	0.03	Smooth and adherent
SnI_4	0.624	0.05	Smooth and bright
$(NH_4)_2SnCl_5$	0.368 (Not completely soluble)	0.04	Smooth, bright, deposit; "treeing" after two minutes
$PbCl_2$	0.278	0.037	Spongy and non-adherent
$Ni(NH_3)_6Br_2$	0.320	0.03	Good, adherent deposit; higher current densities give a black powdery deposit
$Co(NH_3)_5Cl_2$	0.250		No deposit
$K_2Co(CN)_4$	0.332		No deposit
$CoCl_2$		0.02	Smooth deposit; higher current densities give a black powdery deposit
$TiC_2H_5O_2$	0.263	0.025	Some adherent deposit, with marked "treeing"

SnI_4 , $(NH_4)_2SnCl_5$, $PbCl_2$, $Ni(NH_3)_6Br_2$, $CoCl_2$, $TiC_2H_5O_2$ were used for the deposition of the respective metals(16). Here, again, metals higher than zinc in the electromotive series could not be discharged directly from solutions of their salts in acetamide.

The effect of water upon the character of a deposit is strikingly shown in the case of the electrolysis of a solution of zinc cyanide in acetamide. Very poor deposits were obtained using a solution

of zinc cyanide in pure acetamide. The addition of a drop of water to the electrolytic bath caused the formation of bright, adherent deposits, considerably superior to those obtained where pure acetamide solutions had been used.

ELECTRODEPOSITION FROM LIQUID AMMONIA

Perhaps no non-aqueous solvent has attracted so much attention in recent years as liquid ammonia. To quote Franklin (17): "Indeed in all these properties which give water its unique position among solvents liquid ammonia shows a most striking similarity to water. Excepting water itself, it is, so to speak, by far the most water-like substance known. Approaching water as it does in general properties, one might expect to find many resemblances between derivatives of ammonia and the analogous water compounds." Reactions in liquid ammonia have been the subject of considerable study and have led to the adoption by progressive laboratories of methods of synthesis involving liquid ammonia as a solvent.

The study of the electrochemical behavior of solutions of salts in liquid ammonia dates back to the time when interest was first manifested in the nitrogen prototype of water and involves the determination of conductivities (18) of various substances in it. The most important discovery in the course of these investigations was the fact that many organic amides, imides, and nitro compounds yielded conducting solutions. These substances had been regarded as non-electrolytes in aqueous solution, yet in liquid ammonia they were found to behave in many instances as strong electrolytes. In other respects conductivity phenomena were found to be similar to those observed in aqueous solution.

Attempts were made at an early date to prepare free ammonium (19) by the electrolysis of solutions of ammonium salts in liquid ammonia, but none of these experimental efforts have ever yielded any conclusive results. More success attended the electrolysis of solutions of quaternary ammonium salts. The remarkable solubility of the alkali and the alkaline earth metals to give the characteristic blue solutions undoubtedly caused Palmaer (20) to investigate the behavior of tetramethylammonium

and tetraethylammonium chlorides upon electrolysis. He noted the appearance of the blue color in the neighborhood of the cathode and concluded that the free quaternary radicals were discharged. Kraus (21) subsequently confirmed these results and in addition prepared several free mercury alkyl radicals, *viz.*, HgCH_3 , HgC_2H_5 , HgC_3H_7 , by electrolysis of the corresponding iodides or chlorides at temperatures as low as -60° . In the case of these substances Kraus says, "Ammonia not only makes it possible to carry out electrolyses at temperatures favorable to stability, but it also provides a solvent which, even with the most electropositive metals, reacts at a very slow rate."

Not until the past year has the problem of electrodeposition from ammonia been studied intensively. Although investigations involving electrolytic corrosion, specifically nitridation (22) of metallic anodes in liquid ammonia, have been carried out, it was not until Groening and Cady (23) determined decomposition potentials of salts and metal overvoltages in liquid ammonia, that any interest was manifested in the electrodeposition of metals. The decomposition potentials were found, in general, to be of the same order of magnitude as those in water, but the metal overvoltages were found to be somewhat higher. They did not concern themselves with the type of deposit obtained. Taft and Barham (24) took up this problem in some detail to determine the effect of such factors as current density, concentration, acidity, nature of anion, temperature, addition agents and metal base, upon the type of deposit obtained in the electrolysis of solutions of various lead, cadmium, silver, nickel and zinc salts [$\text{Pb}(\text{NO}_3)_2$, $\text{Pb}(\text{C}_2\text{H}_5\text{O}_2)_2$, $\text{Ni}(\text{NH}_3)_6(\text{NO}_3)_2$, $\text{Cu}(\text{NH}_3)_4(\text{NO}_3)_2$, AgNO_3 , $\text{Zn}(\text{NO}_3)_2$]. The current densities necessary for the best deposits were found to be lower in liquid ammonia than in aqueous solutions, except in the cases of zinc and silver. Taft and Barham advocate particularly the possible practical application of liquid ammonia solutions in the deposition of cadmium and lead. (See table 5.)

This same problem has also been studied in a qualitative fashion in the author's laboratory (25). An effort was made here to cover as wide a range of metals as possible by judicious choice of

compounds. (See table 6.) In addition to the metals mentioned by Taft and Barham, mercury, thallium, tin and cobalt plates were also obtained. Cyanides were found to be especially soluble in liquid ammonia and were used in the deposition of zinc and cadmium. The hydrated nitrates of zinc and cobalt were found to give deposits of the respective cations from liquid ammonia. As was expected, electrolysis of soluble alkali metal salts¹ brought about discharge of the free metals with formation of the characteristic blue solution. The persistence of the blue cathodic

TABLE 5
Electrodeposition from liquid ammonia

Conditions for the formation of "best" deposits (TAFT AND BARHAM: J. Phys. Chem. 34, 952 (1930)): temperature, boiling point of solution; anion, nitrate.

METAL	METAL BASE	CURRENT DENSITY PER SQUARE DECIMETER	NORMALITY	ACIDITY PER 100 CC. OF SOLVENT
		amperes		grammes
Pb.....	Fe	0.61	1.0	1.0
Ni.....	Ni	0.15	0.14	0
Cd.....	Fe	1.21	0.5	10
Cu.....	Fe	3.3	0.5	5
Ag.....	Fe	0.92	0.23	0
Zn.....	Fe	1.9	0.53	2

solution is dependent upon the activity of the alkali metal. Blue sodium solutions are quite stable, whereas the similar solution formed as the result of the discharge of cesium disappears shortly after electrolysis is discontinued. The conclusion was reached from these experiments that "the phenomena of electrodeposition of metals from liquid ammonia solutions of their salts closely parallel the behavior of the corresponding aqueous solutions."

Perhaps the most striking advance in recent months was the announcement by Booth (26) and his coworkers that they had

¹ In the case of sodium nitrate and sodium nitrite the alkali metal primarily discharged reacts with the solute to give a yellow precipitate. Analysis of this substance indicates that its composition agrees with the formula Na_2NO_3 , sodium hydronitrite. See MAXTED: J. Chem. Soc. 111, 1016 (1917); also ZINTL AND KOHN: Ber. 61B, 189 (1928).

TABLE 6
Electrodeposition of metals from liquid ammonia
 Temperature, boiling point of solution; metal base, copper

SALT USED	CONCEN- TRATION PER 50 CC.	SOLUBILITY	CURRENT DENSITY PER SQUARE CENTI- METER	CHARACTER OF DEPOSIT
	grams		amperes	
Cu ₂ Cl ₂	Satu- rated	Slightly sol- uble	0.025	Fair
CuBr ₂	Satu- rated	Moderately soluble	0.1	Smooth and adherent
AgNO ₃	5	Very soluble	{ 0.015 0.09	Mottled Smooth and adherent
Zn(NO ₃) ₂ ·6H ₂ O.....	5	Very soluble	{ 0.06 0.55	Smooth and adherent Crystalline; formation of tree-like growths
Zn(CN) ₂	3	Very soluble	{ 0.02 0.11	Uneven Smooth
CdI ₂	Satu- rated	Slightly sol- uble	{ 0.04 0.14	Uneven Smooth and adherent
Cd(CN) ₂	Satu- rated	Moderately soluble	0.04	Smooth deposition. Con- tinued electrolysis caused formation of Cd crystals
HgI ₂	Satu- rated	Moderately soluble	0.04	Hg is discharged. Cu dis- places Hg directly from solution
TlI.....	Satu- rated	Moderately soluble	{ 0.04 0.14	Crystalline and tree-like, mossy
TlNO ₃	2	Very soluble	{ 0.04 0.14	Dendritic and crystalline
SnCl ₄	Satu- rated	Slightly sol- uble	0.025	Excellent deposition
SnI ₄	Satu- rated	Slightly sol- uble	0.14	
Pb(NO ₃) ₂	5	Very soluble	{ 0.03 0.2	Excellent deposit. Con- tinued electrolysis causes the deposit to scale off Crystalline and uneven Smooth; continued elec- trolysis results in the ap- pearance of crystalline growths
Co(NO ₃) ₂ ·6H ₂ O.....	Satu- rated	Very soluble	{ 0.016 0.045	Smooth and adherent Gives a metallic deposit which on contact with air turns black. (Possible formation of an active form of cobalt)

succeeded in depositing beryllium from liquid ammonia and related solvents. Their success attended the preparation of anhydrous beryllium nitrate by dehydration of the hydrated salt in fused ammonium nitrate. By the addition of ammonium nitrate to the solvent they were able not only to decrease the vapor pressure of the solution to such an extent as to make possible electrolysis at room temperatures, but to increase the solubility of the solute as well. Their investigations covered the electrolysis of many different salts in liquid ammonia and publication of these data is awaited with a great deal of interest.

With the manufacture of liquid ammonia in huge quantities and at reasonable prices, its use as a solvent is bound to find practical application. Certainly it has demonstrated its value as an electrolytic medium and its further application along this line awaits only a more extended investigation of its possibilities. Not only the electrodeposition of metals, but cathodic reduction of organic compounds, should be studied in more detail. In consideration of the solubility and conductivity of many organic compounds in liquid ammonia, a study of electro-organic syntheses in this solvent should be particularly fascinating.

DEPOSITION FROM MISCELLANEOUS SOLVENTS

A great many attempts have been made to deposit aluminum electrolytically, but not one of the proposed methods has thus far proven to be of any commercial value. As early as 1902 Plotnikov (27) succeeded in depositing aluminum from a solution of the bromide in ethyl bromide. He also found aluminum bromide to dissolve in toluene, benzene, nitrobenzene, and carbon disulfide to give conducting solutions, but was not successful in depositing the metal from any of these. His experiments were subsequently confirmed by Patten and Mott (28) and other investigators.

A British patent covers the use of acetonitrile (29) as a solvent for the electrodeposition of aluminum. The specifications call for 10 per cent solution of aluminum chloride in this solvent and suggest the use of potassium acid fluoride or phosphorus trifluoride to the extent of about 1 per cent to increase the conductance.

Electrolysis is permitted to take place at temperatures anywhere from 0–100° and the most favorable current density is given as 2–3 amperes per square decimeter.

A U. S. patent issued to G. L. Williams (30) covers the use of aluminum sulfate dissolved in oleum. This suggestion is interesting, even if not practical, from a consideration of 100 per cent sulfuric acid as an acidic solvent of the same type as acetic and formic acids. Sulfuric acid should, therefore, constitute the parent substance of a solvent system in the same sense as we now speak of ammono, aquo and thio compounds. There is only one slightly disturbing feature connected with the patent specifications, since Davidson (31) reports anhydrous aluminum sulfate as absolutely insoluble in 100 per cent sulfuric acid.

The most recent method for the deposition of aluminum makes use of solutions of aluminum alkyl derivatives such as diethyl aluminum iodide and ethyl aluminum diiodide in dry ether (32). The stringent conditions under which this process must be carried out make its practical application of questionable value. However, from a purely scientific point of view this method offers a new line of attack for many other elements which have not yet been obtained even in the metallic form.

Evans (33) has reported the formation of a cathodic deposit of magnesium upon electrolysis of various Grignard solutions. Similar results have also been obtained by Drane and French (34) who used solutions of isoamyl magnesium chloride in ether.

Various alcohols have been tried as electrolytic media. In fact, a process (35) for obtaining metallic lithium by electrolysis of solutions of lithium chloride in ethyl, propyl, butyl and amyl alcohols has been patented. In this connection it is interesting to note that Carrara (36) studied the decomposition potential of sodium ethylate in ethyl alcohol.

In a series of researches being carried out in the author's laboratory at the present time, a whole variety of solvents has been investigated in an attempt to electrodeposit the rare earth metals. Of the fifteen elements which are included in this group only five have ever been prepared in the metallic form and of these only two, cerium and lanthanum, have been obtained in the

pure state. The very fact that the rare earths resemble aluminum, beryllium and barium in their chemical properties makes the problem one which combines the objectionable features of all of these.

Not only have these metals never been deposited from solution, but attempts to prepare amalgams of the rare earths electrolytically have failed (37). Although no marked success has, as yet, been attained in the actual deposition of the rare earth metals from various non-aqueous solvents, no difficulty was encountered in the preparation of rare earth amalgams. Thus, electrolysis of a saturated solution of anhydrous neodymium chloride in absolute ethanol at a current density of 0.06 amperes per square centimeter using a mercury cathode yielded after 10 hours a 2.1 per cent neodymium amalgam (38). Electrolysis of saturated aqueous solutions at higher current densities (0.4-0.6 amperes per square centimeter) also results in the formation of amalgams, but of lesser concentration. Lanthanum amalgams (39) have been prepared in this way, but the preparation of amalgams from aqueous solutions is always accompanied by formation of a heavy precipitate of the basic chloride, when the chloride is employed as solute. It would seem, therefore, that the preparation of amalgams by electrolysis of solutions in non-aqueous solvents possesses certain advantages, especially when a more concentrated product is desired and side reactions are to be avoided.

An interesting solvent which has been found to behave in a rather curious manner is phosphorus oxychloride. Metallic potassium has been discharged cathodically from a solution of potassium iodate in this solvent (40).

Increased interest in non-aqueous solvents has naturally brought about an attempt to classify these on the basis of some common variable. For instance, the suitability of a solvent as an ionizing medium was once believed to be dependent upon its dielectric constant. This idea has long been discarded, for it has been found that given the proper solute practically any substance may act as an ionizing medium. One of the most satisfactory means of differentiating solvents has recently been

given renewed prominence by Hall and Conant (41) and depends upon the affinity of a substance for the proton or hydrogen ion. From this point of view ammonia, because of its pronounced affinity for the hydrogen ion in the formation of the ammonium ion, is regarded as a basic solvent. Water has a lesser affinity for the proton and exhibits a smaller tendency to form the hy-

TABLE 7
Electrodeposition from miscellaneous solvents

METAL	SOLUTE	SOLVENT	REFERENCE
Li.....	LiCl	Methyl, ethyl, propyl, butyl, amyl alcohol	Patten and Mott
Ag.....	AgNO ₃	Acetonitrile	Müller
		Aniline	Kahlenberg
		Benzonitrile	Kahlenberg
		Quinoline	Kahlenberg
Sb.....	SbCl ₃	Methyl alcohol	Kahlenberg
Bi.....	BiCl ₃	Methyl alcohol	Kahlenberg
	AlBr ₃	Ethyl bromide	Plotnikov
	AlCl ₃	Acetonitrile	Lalbin
Al.....	Al ₂ (SO ₄) ₃ (C ₂ H ₅) ₂ AlI C ₂ H ₅ AlI ₂	Sulfuric acid Ether	Williams Keyes, Swann, Klambunde and Schicktanz
Mg.....	Magnesium in ether	Grignard solutions	Evans
K.....	KIO ₃	Phosphorus oxy- chloride	Drane and French Cady and Taft
Sb.....	SbCl ₃	Glacial acetic acid	Nelson, Smith and Audrieth
Bi.....	BiCl ₃		
Sn.....	SnCl ₄		
As.....	AsCl ₃		
Nd amalgam.....	NdCl ₃	C ₂ H ₅ OH	Jukkola and Audrieth

droxonium ion, and consequently is regarded as less basic. Glacial acetic acid from this point of view is regarded as a very acidic solvent. Both Hantzsch and Walden (42) have also pointed out that 100 per cent nitric acid, as well as pure sulfuric acid, may be considered an acidic solvent. The investigations of Hammett (43) and his students seem to indicate that anhydrous formic acid is also a very acidic solvent.

On the basis of this classification of solvents and from a previous study of the electrodeposition of metals from liquid ammonia, an investigation of the behavior upon electrolysis of solutions of salts in glacial acetic acid (44) was undertaken in the author's laboratory. Ammonia and substituted amines are rather distinctive, since they permit electrolytic discharge of the alkali and alkaline earth metals. These elements cannot ordinarily be discharged from more acidic solvents. Compounds of such metals as antimony, bismuth, arsenic and tin undergo considerable solvolysis in more basic media, consequently the use of an acidic solvent such as glacial acetic acid suggested itself for the deposition of those elements which in their chemical properties lie on the borderline between metals and non-metals.

The chlorides of tetravalent tin and of antimony, arsenic, titanium, germanium, and bismuth were found to be appreciably soluble in glacial acetic acid. However, only in the cases of arsenic, antimony, tin, and bismuth was it possible to obtain deposits of the respective metals.

Particularly gratifying were the deposits obtained in the case of antimony and arsenic. Fairly concentrated solutions had to be employed to make them appreciably conducting. Due to the high resistivity of the electrolytic bath some difficulty was encountered in controlling the temperature.

Solutions of antimony trichloride (40–50 grams SbCl₃ per 100 cc. of HC₂H₃O₂) when electrolyzed at low current densities (0.001–0.003 amperes per square centimeter) gave bright deposits upon copper and brass. The addition of water was found to increase the conductivity of the solutions up to the point where more water caused formation of a precipitate. Although the antimony plates obtained were fairly adherent, bending and vigorous treatment caused the metal deposit to peel and dust off.

Good deposits of arsenic were obtained upon electrolysis of a solution of arsenic trichloride (60 grams of AsCl₃ per 100 cc. of glacial acetic acid) at a current density of 0.005–0.006 amperes per square centimeter. The use of higher temperatures did not cause the formation of burnt plates as in the case of antimony.

CONCLUSION

It is a rather striking fact that the subject of electrodeposition from non-aqueous solvents has not been given more prominence until comparatively recent times. The experimental work which has been noted in the literature would warrant, it seems, a more extended investigation. There is no doubt that the publications of Franklin and Kraus dealing with liquid ammonia, those of Walden dealing with such solvents as liquid sulfur dioxide, acetonitrile, etc., those of Maass concerning hydrogen peroxide, those of Davidson, Conant and Hall concerning acetic acid, and many others also have opened up a prolific field of experimental endeavor and have spurred investigations in all phases of non-aqueous solvent chemistry.

Many factors must be considered in undertaking investigations which deal with the electrodeposition of metals from non-aqueous solvents. Unfortunately, no satisfactory criteria are at hand to enable the ready choice of solute and solvent for the discharge of any one particular ion. However, it is to be hoped that some generalization may still be applied to the isolated and fragmentary information at our disposal, so that we may be able to predict what solvent and solute to employ to bring about discharge of any element in the periodic table. The possible realization of such an aim should be sufficient incentive for a more thorough and extended study of electrodeposition from non-aqueous solvents.

REFERENCES

- (1) CONANT AND HALL: J. Am. Chem. Soc. **49**, 3062, 3068 (1927).
- (2) LASZYNSKI: Z. Elektrochem. **2**, 55 (1895-96).
- (3) LASZYNSKI AND GORSKI: Z. Elektrochem. **4**, 292 (1897).
- (4) KAHLENBERG: J. Phys. Chem. **3**, 602 (1899).
- (5) KAHLENBERG: J. Phys. Chem. **4**, 394 (1900).
- (6) MÜLLER AND OTHERS: (a) Monatsh. **43**, 67 (1922); (b) ibid. **43**, 75 (1922); (c) ibid. **43**, 419 (1922); (d) ibid. **44**, 219 (1923); (e) ibid. **45**, 525 (1924).
- (7) HEVESY: Z. Elektrochem. **16**, 672 (1910).
- (8) COHEN: Ber. **37**, 811 (1904).
- (9) PIERLE: J. Phys. Chem. **23**, 517 (1919).
- (10) PATTEN AND MOTT: Trans. Am. Electrochem. Soc. **15**, 529 (1909).
- (11) PATTEN AND MOTT: J. Phys. Chem. **8**, 483 (1904).
- (12) PATTEN AND MOTT: J. Phys. Chem. **12**, 49 (1908).

- (13) SIEMENS: *Z. anorg. Chem.* **41**, 249 (1904).
- (14) WALDEN: *Z. physik. Chem.* **46**, 103 (1903); **54**, 129 (1905); **55**, 683 (1906).
- (15) RÖHLER: *Z. Elektrochem.* **16**, 419 (1910).
- (16) YNTEMA AND AUDRIETH: *J. Am. Chem. Soc.* **52**, 2693 (1930).
- (17) FRANKLIN: *J. Am. Chem. Soc.* **46**, 2137 (1924).
- (18) (a) FRANKLIN: *J. Am. Chem. Soc.* **27**, 191 (1905).
(b) KRAUS: *Am. Chem. J.* **23**, 277 (1900); **24**, 83 (1901).
(c) SMITH: *J. Am. Chem. Soc.* **49**, 2162 (1927).
- (19) RUFF: *Ber.* **34**, 2804 (1901).
MOISSAN: *Compt. rend.* **133**, 715 (1901).
- (20) PALMAER: *Z. Elektrochem.* **8**, 729 (1902).
- (21) KRAUS: *J. Am. Chem. Soc.* **35**, 1732 (1913).
- (22) BROWNE, HOLMES AND KING: *J. Am. Chem. Soc.* **41**, 1769 (1919).
- (23) GROENING AND CADY: *J. Phys. Chem.* **30**, 1597 (1926).
- (24) TAFT AND BARHAM: *J. Phys. Chem.* **34**, 929 (1930).
- (25) AUDRIETH AND YNTEMA: *J. Phys. Chem.* **34**, 1903 (1930).
- (26) BOOTH: *J. Am. Chem. Soc.* **52**, 2581 (1930).
- (27) PLOTNIKOV: *J. Russ. Phys. Chem. Soc.* **34**, I, 466 (1902).
- (28) PATTEN AND MOTT: *J. Phys. Chem.* **8**, 548 (1904).
- (29) British pat. 106,400 to E. L. Lalbin. Cf. C. A. **11**, 2563 (1917).
- (30) WILLIAMS, G. L.: U. S. pat. 1,351,144. Cf. C. A. **14**, 3200 (1920).
- (31) DAVIDSON: *J. Am. Chem. Soc.* **47**, 968 (1925).
- (32) KEYES, SWANN, KLABUNDE AND SCHICKTANZ: *Ind. Eng. Chem.* **20**, 1068 (1928).
- (33) EVANS: Report given at the meeting of the American Chemical Society in Cincinnati, September 1930.
- (34) DRANE AND FRENCH: *J. Am. Chem. Soc.* **52**, 4904 (1930).
- (35) PATTEN AND MOTT: *J. Phys. Chem.* **8**, 153 (1904).
- (36) CARRARA: *Gazz. chim. ital.* **38**, I, 698 (1908).
- (37) KETTENBEIL: *Z. anorg. Chem.* **38**, 213 (1904).
- (38) JUKKOLA, E. E. AND AUDRIETH, L. F.: Unpublished work.
- (39) MEINTS, R. E. AND AUDRIETH, L. F.: Unpublished work.
- (40) CADY AND TAFT: *J. Phys. Chem.* **29**, 1057, 1068 (1925).
- (41) HALL AND CONANT: *J. Am. Chem. Soc.* **49**, 3047, 3062 (1927). See also *J. Chem. Education* **7**, 782 (1930).
- (42) HANTZSCH AND WALDEN: *Z. Elektrochem.* **29**, 228 (1923); *Ber.* **58**, 620, 955 (1925); *Z. anorg. Chem.* **29**, 383 (1902); *Z. angew. Chem.* **37**, 390 (1924).
- (43) HAMMETT AND DIETZ: *J. Am. Chem. Soc.* **52**, 4795 (1930).
- (44) NELSON, H. W., SMITH, L. H., AND AUDRIETH, L. F.: Unpublished work.

POLYMERIZATION¹

WALLACE H. CAROTHERS

*Experimental Station, E. I. du Pont de Nemours and Company, Wilmington,
Delaware*

Received March 21, 1931

TABLE OF CONTENTS

I. Definitions	354
1. Current definitions	354
2. Proposed definitions	355
3. Linear and non-linear polymers	356
4. Types of compounds capable of polymerizing	356
5. Types of polymerization	357
6. Condensation polymerizations and bifunctional reactions	358
II. Condensation polymerization	359
1. Polyesters	359
a. The self-esterification of hydroxy acids	359
b. Polyesters from dibasic acids and glycols	361
2. Bifunctional Wurtz reactions and Friedel Crafts reactions	368
3. Other bifunctional reactions	369
a. Polyamides	370
b. Polyamines	370
c. Polyacetals	371
d. Polyanhydrides	371
e. Grignard reactions	372
f. Sulfur and selenium compounds	373
g. Miscellaneous	374
4. Stereochemical factors involved in condensation polymerization	376
a. Large rings	380
III. Polymerization involving cyclic compounds	383
1. Six-membered cyclic esters	383
2. Adipic anhydride	388
3. Diketopiperazines and polypeptides	389
4. Ethylene oxide	391
IV. Addition polymerization of unsaturated compounds	392
1. Ethylene and other olefines	393
2. Vinyl compounds	394
3. Dienes	399
4. Aldehydes	401

¹ Studies on polymerization and ring formation. IX. Communication No. 55 from the Experimental Station of the E. I. du Pont de Nemours and Company.

V. Polyfunctional reactions and non-linear polymers	402
VI. Natural polymers	404
1. The association theory versus the structural theory	404
2. Cellulose	406
3. Rubber	408
VII. The physical properties of high polymers	412
1. Solubility and colloidal properties	413
2. Crystallinity	415
3. Mechanical properties	418

This review is concerned with reactions that result in the combination of simple molecules to form materials of high molecular weight, and especially with the nature of such reactions and the structure and properties of the products. For the sake of comparison, the structures of certain natural polymers concerning which it is possible to make definite statements are briefly considered also. The literature on the subject of high polymers is most profuse, and from the theoretical side it has until very recently been contradictory and confused. For these reasons the present discussion is of necessity rather selective and critical. (For other general discussions of certain aspects of this field the reader may consult references 1 to 7 and 18.) It is necessary also to define especially the sense in which certain terms are to be used.

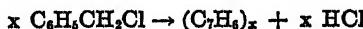
I. DEFINITIONS

1. Current definitions

It is generally agreed by organic chemists (9) that polymerization is chemical combination involving the operation of primary valence forces, and that the term polymer should not be used (as it frequently is by physical and inorganic chemists) to name loose or vaguely defined molecular aggregates. Beyond this, however, there is not much agreement. The word polymer was introduced by Berzelius (12) nearly a century ago to recognize the fact that two compounds may have the same composition but different molecular weights, and he classified polymerism as a special type of isomerism. The accepted meaning of these terms has subsequently undergone considerable change, but current textbook definitions always state that a monomer and its polymer have the same composition, and it is usually either stated or implied that the process of polymerization is peculiar to unsaturated compounds and consists in self-addition. But recently discovered facts show that these conditions really are not satisfied in many instances to which the terms polymer and polymerization

are applied. The formula of α -polyoxymethylene, for example, is not $(\text{CH}_2\text{O})_x$, but $(\text{CH}_2\text{O})_x\text{H}_2\text{O}$ (110).

It is true also that many compounds which are not unsaturated are capable of reacting with themselves to form products of high molecular weight, and that such reactions are quite commonly called polymerization. For example, benzyl chloride in the presence of aluminum chloride:



One can assume that the first step in this reaction is the elimination of HCl to yield the radical $\text{C}_6\text{H}_5\text{CH}=$, which, being unsaturated, is capable of reacting with itself by addition. On the other hand, it may be that this is simply a polymolecular Friedel Crafts reaction, the first step being the formation of $\text{C}_6\text{H}_5\text{CH}_2\text{-C}_6\text{H}_4\text{CH}_2\text{Cl}$, which is capable of reacting with itself by the same mechanism and finally yielding a very long chain in which the chlorine content is negligible. If the usual definitions are adopted, this reaction can properly be called polymerization only if it proceeds by the first mechanism. Curiously enough, one actually finds in the literature cases in which the fact that a reaction is called polymerization is used to prove that the first step must be the formation of an unsaturated intermediate capable of reaction with itself by addition. It is perhaps unnecessary to say that the questions of the composition of high polymers and the mechanism of their formation are frequently rather intricate and cannot be solved in advance by definition.

2. Proposed definitions

It is more practical and useful (and also more consistent with actual usage) to define polymerization as any chemical combination of a number of similar molecules to form a single molecule. A polymer then will be any compound that can be formed by this process or degraded by the reverse process: formaldehyde can be regenerated from polyoxymethylene by the action of heat; ethylene glycol can be obtained from polyethylene glycol by hydrolysis; cellulose can be hydrolyzed to glucose; and rubber can be formed by the reaction of isoprene with itself.

3. Linear and non-linear polymers

The simplest and perhaps the most numerous and important high polymers are characterized structurally by the fact that their molecules are long chains built up from a repeating radical or unit. This type of structure may be represented by the general formula,



The repeating radical —A— is called the *structural unit*. The number and nature of these units determine the nature of the molecule. To complete this formula it is necessary to specify the disposition of the terminal valences; they may conceivably be mutually joined to form a cyclic structure or saturated by univalent groups. Whether the chain is open or closed, polymers of this class will be called *linear polymers*. It should be added that linear high polymers are usually mixtures containing chains of different lengths.

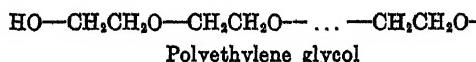
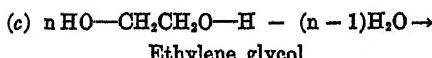
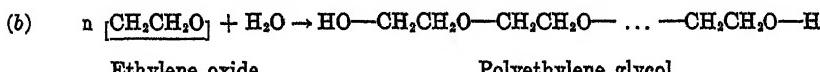
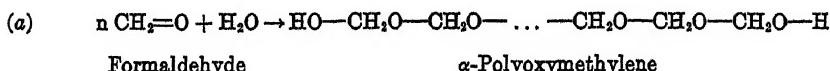
If the polymer is derived from a single compound the structural units of the chain will in general be identical, but the mutual reaction of two or more compounds that are chemically similar but not identical may lead to chains made up from two or more different units. Such products will be called linear mixed polymers.² Many polypeptides and proteins belong to this class. Non-linear polymers also exist; they can be formed, for example, by the cross linking of long chains into two- or three-dimensional structures.

4. Types of compounds capable of polymerizing

The step-by-step synthesis of long molecular chains containing a repeating unit is illustrated by Fischer's famous synthesis of polypeptides. Reactions of polymerization, however, lead to the formation of polymeric chains in a single operation. The capacity for self-combination of this kind is found among simple molecules of three different types: (a) unsaturated compounds;

² The term heteropolymer has also been suggested (23).

(b) cyclic compounds; (c) polyfunctional compounds. These are illustrated by the following examples.



In each of these examples the product molecule is made up of a repeating series of identical units. Each unit corresponds with one molecule of the starting material. The latter, therefore, is called the monomer (*mer* = part). Ethylene glycol and ethylene oxide are both monomers since, although they differ in structure and composition, their molecules contain one $-\text{CH}_2\text{CH}_2\text{O}-$ unit each. Similarly diethylene glycol, $\text{HO}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{H}$, is a dimer.

5. Types of polymerization

Molecules can combine to form larger molecules either by addition or by condensation,⁴ and two corresponding types of polymerization may be recognized. The formation of polyoxymethylene from formaldehyde is addition or *A* polymerization. The formation of polyethylene glycol directly from ethylene glycol

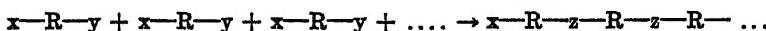
³ The direct polyintermolecular dehydration of ethylene glycol to polyethylene glycol not involving the intermediate formation of ethylene oxide is perhaps somewhat hypothetical. It is used here merely as a formal illustration.

⁴ The term condensation is used here to name any reaction that occurs with the formation of a new bond between atoms not already joined and proceeds with the elimination of elements (hydrogen, nitrogen, etc.) or of simple compounds (water, ethyl alcohol, ammonia, sodium bromide, etc.). Examples are the Wurtz reaction, Friedel Crafts reaction, esterification, etc. See Kempf in Houben-Weyl, ref. 6, Volume II, p. 717.

is condensation or *C* polymerization.⁵ This second type of polymerization, although its existence as a general phenomenon has only recently been recognized (18), is especially simple both practically and theoretically since it involves the known behavior of typical functional groups.

6. Condensation polymerizations and bifunctional reactions

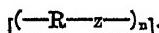
A general class of condensation polymerizations is represented by the equation



In the formula $x-R-y$, R is a bivalent radical and x and y are functional groups capable of reacting with each other to form the known functional group z. Thus if x is HO and y is COOH, z will be CO—O. The compounds $x-R-y$ are called bifunctional compounds and their reactions, bifunctional reactions. Reactions of the type, $x-R-x + y-R'-y \rightarrow product$, may be included in this class.

Bifunctional reactions present the possibility of following various courses. (a) They may be intramolecular at the first stage. The product will then be the cyclic monomer

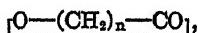
(b) Reaction may be intermolecular at the first stage and intra-molecular at some subsequent stage. The product will then be a cyclic polymer



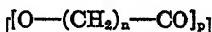
(c) Reaction may be exclusively intermolecular. In this case the product will be an open chain of the type $x-R-z-R-z\dots-R-z-R-y$. These possibilities may be illustrated

⁵ The term condensation polymerization was at one time applied by Staudinger (38) to addition polymerizations that involve the migration or displacement of a group or an atom, but its use in this sense has never become general. In view of its obvious propriety for the purpose, it was adopted by the writer (18) as a name for polyintermolecular condensation.

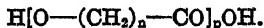
by the hydroxy acids of the series $H—O—(CH_2)_n—CO—OH$. The self-esterification of these might lead to the simple lactones



or to cyclic polyesters



or to open chain polyesters



All three of these possibilities can be realized.

II. CONDENSATION POLYMERIZATION

1. Polyesters

Bifunctional esterifications are especially suitable for the study of condensation polymerization, because esterification is a reversible reaction, and it is entirely free from side reactions under conditions that are easy to realize.

a. The self-esterification of hydroxy acids. It is well known that hydroxy acids react with themselves to form cyclic esters when there is the possibility of forming a five- or a six-membered ring: γ - and δ -hydroxy acids lead to the corresponding lactones, and α -hydroxy acids yield the cyclic dimers. Thus glycolic acid reacts with itself to form glycolide:⁸



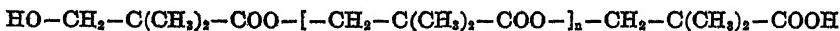
The γ -lactones are stable substances, but the δ -lactones and glycolide and its analogs on being heated are rapidly transformed into polyesters of high molecular weight. This peculiar behavior of the six-membered cyclic esters is considered in more detail in a later paragraph.

⁸ But it is by no means certain that the glycolide is ever formed through the steps glycolic acid \rightarrow glycolylglycolic acid \rightarrow glycolide. Dietzel and Krug (19) have shown that in the self-esterification of lactic acid the primary steps are: lactic acid \rightarrow lactyllactic acid \rightarrow polylactyllactic acids. Lactide when it is formed results from the depolymerization of these polylactyllactic acids.

No information is available concerning the behavior of the simplest ϵ -hydroxy acid, hydroxycaproic acid, but the corresponding bromo acid when treated with sodium ethylate in alcohol solution gives a poor yield of the seven-membered lactone (37). The chief product is an undistillable material, undoubtedly polyester. ϵ -Hydroxycaprylic acid, $C_2H_5\text{—CHOH—}(CH_2)_4\text{—COOH}$ is partly converted into the corresponding seven-membered lactone on being heated, but the next member of this series, $C_2H_5\text{—CHOH—}(CH_2)_5\text{—COOH}$, under the same conditions gives only an undistillable residue (24).

Larger lactones containing rings of fourteen to eighteen atoms are known (25), and also β -lactones (four-membered rings) (26, 27). All of these are fairly stable compounds, but none of them has ever been prepared directly from the corresponding acid.⁷

The attempt to prepare self-esters from simple β -hydroxy acids results merely in dehydration to the unsaturated acid, but in hydroxypivalic acid, $\text{HO—CH}_2\text{—C(CH}_3)_2\text{—COOH}$, this usual behavior is impossible because of the absence of any α -hydrogen. The self-esterification of this acid has been studied by Blaise and Marcilly (28). It occurs at 200°C. or at lower temperatures in the presence of certain catalysts. The product is a microcrystalline powder insoluble in most organic solvents. It forms sodium salts insoluble in cold water, and it is readily hydrolyzed to hydroxypivalic acid. These facts clearly indicate a structure of the type



and on the basis of molecular weight and analytical data Blaise and Marcilly assign to n the value 4.

⁷ Kerschbaum (36) records various attempts to prepare the lactone of ambröttolic acid (hexadecene-7-ol-16-acid-1) from the acid obtained by hydrolyzing the naturally occurring lactone. By heating the acid he obtained a small amount of oil which, from its odor, was inferred to contain lactone. The chief product, however, was a non-volatile material soluble in alkali. Neither Chuit and Haussner (29) nor Lycan and Adams (30) report any evidence for the presence of lactone in the self-esters prepared from higher ω -hydroxy acids. Unpublished experiments made in this laboratory have failed to reveal the presence of any lactone among the products of the dehydration of ω -hydroxypentadecanoic acid.

In regard to the higher ω -hydroxy acids, Chuit and Haussner (29) have recently synthesized the entire series from HO—(CH₂)₇—COOH to HO—(CH₂)₂₀—COOH. All these acids readily undergo self-esterification on being heated. The products are not the corresponding lactones (which in several cases are known), but solid acidic materials whose properties are consistent with the general formula HO—R—CO—O—R—CO—...—O—R—COOH. Similar products obtained by the self-esterification of higher hydroxy acids have also been investigated by Bougalt and Bourdier (31) and by Lycan and Adams (30).

The presence of a terminal carboxyl group in each of these products proves that the ester formation is exclusively intermolecular. It should be possible, therefore, by regulating the degree of completeness of the esterification to obtain molecules of various lengths and, in particular, to obtain exceedingly long molecules. Such molecules are of especial interest in connection with the study of high polymers, since the nature of the acid used absolutely determines the structural unit of the polyester.

b. Polyesters from dibasic acids and glycols. The study of bi-functional esterifications from this standpoint was first undertaken by the writer and his collaborators (32 to 35). Dibasic acids and glycols were used as starting materials since these are more readily accessible than hydroxy acids, and they also permit more numerous structural variations in the ester product.

Esters derived from dibasic acids and glycols can be prepared by any of the typical reactions ordinarily used in the preparation of simple esters, e.g.:

- (a) HO—R—OH + HOOC—R'—COOH → —O—R—O—CO—R'—CO— + H₂O
- (b) HO—R—OH + EtOOC—R'—COOEt → —O—R—O—CO—R'—CO— + EtOH
- (c) HO—R—OH + C₆H₅OOC—R'—COOC₆H₅ → —O—R—O—CO—R'—CO— + C₆H₅OH
- (d) HO—R—OH + ClCO—R'—COCl → —O—R—O—CO—R'—CO— + HCl
- (e) Br—R—Br + AgOCO—R'—COOAg → —O—R—O—CO—R'—CO— + AgBr

In these equations —O—R—O—CO—R'—CO— represents the structural unit of the product. The studies have proved that the

nature of the ester is completely determined by the number of atoms in the chain of the unit: if this number is five, the product is monomeric and cyclic (i.e., it contains only one unit); if the number is six, the product can be obtained in both monomeric and polymeric forms and these are interconvertible; if the number is more than six, the product is exclusively polymeric.

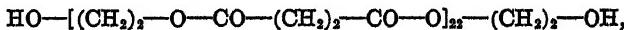
The preparation of an ester of this last class may be illustrated by the action of succinic acid on ethylene glycol.⁸ Succinic acid mixed with a slight excess of glycol is heated in a distilling flask. At about 160–175°C. rapid esterification sets in, accompanied by the liberation of water. When no more water is evolved the temperature is raised to 220–250°C. and the pressure is reduced below 1 mm., whereupon most of the excess glycol distils. The ester product is completely non-volatile and remains in the distilling flask in the form of an exceedingly viscous liquid. When this is cooled it solidifies to a hard, brittle, opaque, white mass. This can be dissolved in cold chloroform and precipitated as a granular powder by the addition of ether or benzene. It can be recrystallized from hot ethyl acetate or a large volume of hot acetone. The recrystallized ester melts at about 102°C. A product having the same physical properties can be prepared in the same way by the action of diethyl succinate or diphenyl succinate on ethylene glycol. The reaction then consists in ester interchange and the volatile product liberated is alcohol or phenol.

By the method of direct esterification or by ester interchange the esters listed in table I have been prepared. They are without exception highly polymeric, the molecular weights being on the average in the neighborhood of 3000. Molecular weights of several of the esters have been determined in a variety of solvents and by both freezing and boiling point methods, and within the limits of experimental error the same values have been found.

⁸ The preparation of ethylene succinate by reaction between the acid and glycol was first carried out by Lourenco (17). Later Davidoff (40) and Vorländer (41) prepared it by various methods, and the latter investigator assigned to it the formula of a sixteen-membered cyclic dimer. His molecular weight determinations were apparently in error. The dimeric ester is now known (42, 34) and its properties are quite different from those of Vorländer's ester, which closely resembles the polymeric ester prepared from the acid and the glycol.

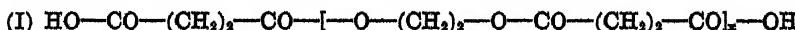
These esters show considerable similarity in their physical properties. They are completely non-volatile and all of them dissolve quite readily in cold chloroform. Those derived from the higher glycols or acids also dissolve in cold benzene, and in the solid state they are less hard and more wax-like than ethylene succinate. In spite of their high molecular weight and lack of complete homogeneity all these esters except the phthalates are crystalline. The melting points vary slightly from one preparation to another of a given ester and usually cover a range of about 2°.

The ethylene succinate described above is neutral, and its composition and molecular weight indicate the average formula⁹



i.e., it is an open chain derived from twenty-two molecules of acid and twenty-three molecules of glycol.

The hydroxyls at the end of this chain do not readily react with the usual reagents for alcohol groups; their sluggish behavior in this respect is characteristic of many high molecular weight materials. However, on being heated to a fairly high temperature with *p*-bromobenzoic anhydride the ester yields a di-*p*-bromobenzoyl derivative identified as such by its bromine content. The ester also reacts with molten succinic anhydride and yields an acidic ester whose neutral equivalent and observed molecular weight agree with the formula I d.



I a, x = 6; I b, x = 9; I c, x = 12; I d, x = 23.

⁹ The molecules of this product certainly do not all have the same length, but on the other hand it appears to be much more nearly homogeneous than any products ever obtained by addition polymerization (polystyrene, etc.). In its qualitative solubility behavior it resembles a chemical individual, and fairly elaborate fractional crystallizations have failed to separate it into portions showing any considerable difference in their properties. The reason for its relatively great homogeneity lies in the conditions of its formation, which are such as to force much smaller molecules to react with themselves, but are not sufficiently drastic to cause the formation of much larger molecules. Under other conditions polymeric mixtures having a wide range of molecular weights can be obtained.

TABLE I
Polyesters from glycols and dibasic acids

COMPOUND	FORMULA OF STRUCTURAL UNIT	PHYSICAL PROPERTIES			
		ATOMS IN CHAIN OF SERVED STRUCTURAL UNIT	AVER-AGE OF MOLECULAR WEIGHT	AVER-AGE NUMBER OF STRUCTURAL UNITS FOR MOLECULE	AVER-AGE NUMBER OF STRUCTURAL UNITS FOR MOLECULE
Tetramethylene carbonate . . .	—O—(CH ₂) ₄ —O—CO—	7	1400	11-12	Microcrystalline powder, m.p. 59°
Pentamethylene carbonate . . .	—O—(CH ₂) ₅ —O—CO—	8	2700	20-22	Microcrystalline powder, m.p. 44-46°
Hexamethylene carbonate . . .	—O—(CH ₂) ₆ —O—CO—	9	2800	18-21	Microcrystalline powder, m.p. 55-60°
Decamethylene carbonate . . .	—O—(CH ₂) ₁₀ —O—CO—	13	1800	8-10	Microcrystalline powder, m.p. 65°
Ethylen succinate	—O—(CH ₂) ₂ —O—CO—(CH ₂) ₂ —CO—	8	3000	20	Microcrystalline powder, m.p. 108°
Ethylen adipate	—O—(CH ₂) ₂ —O—CO—(CH ₂) ₄ —CO—	10	2800	17	Microcrystalline powder, m.p. 60°
Hexamethylene succinate . . .	—O—(CH ₂) ₆ —O—CO—(CH ₂) ₂ —CO—	12	3400	14	Microcrystalline powder, m.p. 67°
Ethylen sebacate	—O—(CH ₂) ₂ —O—CO—(CH ₂) ₈ —CO—	14	4000	13	Microcrystalline powder, m.p. 79°
Trimethylene sebacate	—O—(CH ₂) ₃ —O—CO—(CH ₂) ₈ —CO—	15	3100	12	Microcrystalline powder, m.p. 56°
Decamethylene adipate	—O—(CH ₂) ₁₀ —O—CO—(CH ₂) ₄ —CO—	18	3000	10	Microcrystalline powder, m.p. 77°
Decamethylene sebacate	—O—(CH ₂) ₁₀ —O—CO—(CH ₂) ₈ —CO—	22	3000	8	Microcrystalline powder, m.p. 74°

Ethylene phthalate.....	$-\text{O}-(\text{CH}_2)_2-\text{O}-\text{CO}-\text{C}_6\text{H}_4-\text{CO}-$	8	4800	25	Hard, transparent resin
Trimethylene phthalate.....	$-\text{O}-(\text{CH}_2)_3-\text{O}-\text{CO}-\text{C}_6\text{H}_4-\text{CO}-$	9	3100	14	Soft, transparent resin
Hexamethylene phthalate.....	$-\text{O}-(\text{CH}_2)_6-\text{O}-\text{CO}-\text{C}_6\text{H}_4-\text{CO}-$	12	1800	7	Soft, transparent gum
Decamethylene phthalate.....	$-\text{O}-(\text{CH}_2)_{10}-\text{O}-\text{CO}-\text{C}_6\text{H}_4-\text{CO}-$	16	2100	7	Very viscous, transparent syrup
Trimethylene oxalate.....	$-\text{O}-(\text{CH}_2)_3-\text{O}-\text{CO}-\text{CO}-$	7	2000	15	Microcrystalline powder, m.p. 88°
Hexamethylene oxalate.....	$-\text{O}-(\text{CH}_2)_6-\text{O}-\text{CO}-\text{CO}-$	10	1100	7	Microcrystalline powder, m.p. 66°
Decamethylene oxalate.....	$-\text{O}-(\text{CH}_2)_{10}-\text{O}-\text{CO}-\text{CO}-$	14	1200	6	Microcrystalline powder, m.p. 79°
Polyester from hydroxydecanoic acid.....	$-\text{O}-(\text{CH}_2)_9-\text{CO}-$	11	5000	20	Microcrystalline powder, m.p. 76° (30)

Similar acidic esters of lower molecular weight have also been obtained by partial esterification of glycol with an excess of succinic acid and fractional crystallization of the product. The observed molecular weights and neutral equivalents of the fractions corresponding to various values of x in formula I are indicated in table 2.

The presence of these terminal groups proves that the esterification of succinic acid by ethylene glycol is intermolecular at every stage.¹⁰ The reaction evidently involves a series of con-

TABLE 2
Acidic ethylene succinates

FORMULA	MELTING POINT °C.	MOLECULAR WEIGHT		SODIUM SALT	
		Calculated from neutralization equivalent	Found by ebullioscopy	Melting point °C.	Molecular weight calculated from sodium content
I a.....	73	1020	1070	91	1030
I b.....	82	1340	1380	97	1460
I c.....	90	1800	1580	100	2010
I d.....	98	3400	3110	109	3740

densations resulting in the production of ester molecules of progressively greater length. The first product might be



and the second



Moreover, since both glycols and dibasic acids have a tendency to esterify at both ends simultaneously, it is reasonable to suppose that similar chains will be present, some of which are terminated

¹⁰ Lycan and Adams (30) also present convincing evidence of the open chain structures of polyesters derived from ω -hydroxydecanoic acid. They isolated fractions whose molecular weights estimated by titration with alkali ranged from 1000 to nearly 9000. All of these fractions formed potassium salts which were completely soluble in warm water.

at both ends by carboxyl and others by hydroxyl. It is evident that an exceedingly large number of species may be involved in the ester equilibrium.¹¹ But as the amount of water participating in the equilibrium is diminished by its constant removal, the smaller molecules are forced to couple with each other until finally practically none of them remains. A further simplification of the kinds of molecular species present in the product is effected by using an excess of glycol, since this makes all the terminal groups alike. For this purpose, it makes no difference how large an excess of glycol is used, provided the reaction mixture is finally heated for some time in a high vacuum, since under these conditions the reaction can be propagated by ester interchange. Thus it is possible to isolate the monomeric ester, *bis*- β -hydroxyethylsuccinate,



and this on being heated to 200°C. in a vacuum loses glycol and is converted into the neutral polyethylene succinate of molecular weight 3000 already described. In view of this fact it is obvious that the accidental mutilation of the terminal groups (e.g., the loss of OH or CO₂) cannot in itself prohibit the progress of the coupling; the reaction can progress by ester interchange involving the last ester linkage.

Apparently the ultimate factors that set the attainable limit on the length of polyester molecules are purely physical. As the molecular weight becomes greater, the reaction product becomes more viscous and the rate of diffusion of the volatile product (water or alcohol) to the surface becomes slower and slower.¹² Moreover as the reaction progresses, the mobility of the reacting molecules diminishes and the relative concentration of the reactive groups becomes smaller. All of these factors affect the rate and some of them affect the position of equilibrium, but the molecular weight at which a practical limit is reached will depend upon the

¹¹ The kinetics of the reaction between phthalic anhydride and ethylene glycol have been studied by Kienle and Hovey (43).

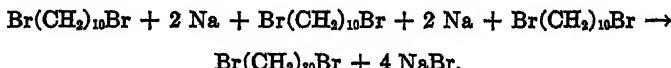
¹² At least in the crystalline state polyesters adsorb water and hold it very tenaciously.

temperature and pressure, the area and thickness of the reacting mass, etc., and by a suitable adjustment of these factors it is possible to obtain polyesters having very much higher molecular weights than those listed in table 1 (51).

2. Bifunctional Wurtz reactions and Friedel Crafts reactions

The action of sodium on polymethylene bromides, $\text{Br}(\text{CH}_2)_n\text{Br}$, leads in certain cases to the formation of cyclic hydrocarbons. Cyclopropane, cyclopentane, and cyclohexane have been prepared in this way. On the other hand, the action of sodium on deca-methylene bromide does not yield any cyclic hydrocarbons.

In the presence of absolute ether this reaction proceeds very smoothly (44, 45). The product, which is for the most part insoluble in ether, consists of a complex mixture formed by the coupling of various numbers of molecules of the halide with each other, e.g.,

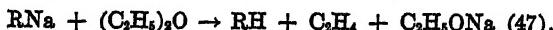


At the same time, owing to the participation of the ether in the reaction, most of the terminal bromine atoms are replaced by hydrogen.¹³ This reduction of the terminal groups can finally be carried to completion by the action of sodium in boiling butyl ether, and the product then consists of a mixture having the general formula

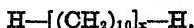


in which the values of x range from 1 to at least 10. The various members of this mixture up to and including $n\text{-C}_{70}\text{H}_{142}$ can be separated and isolated in a state of purity by fractional evaporation in a molecular still followed by crystallization. The identity of these fractions is established by their melting points and their

¹³ In its mechanism the Wurtz reaction undoubtedly involves as its first step the formation of the sodium compound RNa (46). This normally couples with another molecule of the halide, but if ether is present it may be destroyed by the reaction



x-ray diffraction patterns. There is no evidence of the presence of any materials in the mixture not belonging to the series



About 25 per cent of the total product consists of members standing above $\text{C}_{70}\text{H}_{142}$. These are not capable of being distilled, and they cannot be separated from each other by fractional crystallization. The average molecular weight of this material indicates that it must contain hydrocarbons at least as high as $\text{C}_{100}\text{H}_{202}$.

The following results of unpublished studies made by Dr. R. A. Jacobson in this laboratory are more or less closely related to those described above. The action of metallic sodium on *p*-dibromobenzene in absolute ether leads to a product corresponding in composition to the formula $\text{Br}-\text{C}_6\text{H}_4-(\text{C}_6\text{H}_4)_n-\text{C}_6\text{H}_4\text{Br}$. When the reaction is carried out in boiling toluene the product formed corresponds approximately in composition and molecular weight to the formula $\text{Br}-\text{C}_6\text{H}_4-(\text{C}_6\text{H}_4)_{12}-\text{C}_6\text{H}_4\text{Br}$. Both of these products are readily soluble in benzene. *p*-Xylylene bromide, $\text{BrCH}_2-\text{C}_6\text{H}_4-\text{CH}_2\text{Br}$, when treated with sodium in hot toluene, yields a very insoluble hydrocarbon which does not melt below 350°C. Apparently a very large number of *p*-xylylene units participate in this coupling.

Benzyl chloride in the presence of aluminum chloride or ferric chloride (see 10) reacts with itself to form resins of high molecular weight. This is evidently a bifunctional Friedel Crafts reaction involving the progressive coupling of successively longer chains with the elimination of hydrogen chloride. Depending upon the conditions of their formation these resins are fusible and soluble, or infusible and insoluble. Molecular weight values indicate that the fusible resins are formed from fourteen to twenty-five molecules of benzyl chloride. The infusible resins are no doubt much more highly polymeric. Benzyl fluoride in the presence of a trace of acid reacts very vigorously with itself in a similar manner (11).

3. Other bifunctional reactions

The number of possible types of condensation polymers is practically unlimited. Although very few of these possibilities

have received any considerable study, the following examples at least indicate that the formation of such polymers is the usual result of bifunctional reactions when structural features preclude the formation of a five- or a six-membered ring.

a. Polyamides. The acids $\text{NH}_2(\text{CH}_2)_3\text{COOH}$ and $\text{NH}_2(\text{CH}_2)_4\text{COOH}$ are readily dehydrated to the corresponding monomeric lactams (48). There is no record of either of these being caused to polymerize. The next higher member yields two products (49, 50a). One of these (20–30 per cent) is the lactam (seven-membered ring), a distillable crystalline material; the other is a polyamide, an undistillable hard, waxy material insoluble in most solvents except concentrated hydrochloric acid, phenol, and hot formamide. It can be hydrolyzed quantitatively to the amino acid. Molecular weight determinations indicate the presence of at least ten structural units in its molecule. The formation of this polymer is due to direct intermolecular condensation and the formation of the lactam is an independent reaction, for the latter cannot be polymerized under the conditions that lead to the production of the former. The acid $\text{NH}_2(\text{CH}_2)_6\text{COOH}$ on being dehydrated yields exclusively a product that is polymeric (50), although the corresponding monomeric lactam has been prepared by another method and is a stable substance (164). The acid $\text{NH}_2(\text{CH}_2)_{10}\text{COOH}$ also yields only polyamide (51).

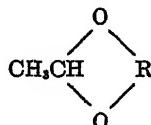
A number of polyamides have been prepared by Dr. J. E. Kirby in this laboratory by the action of dibasic acids on aliphatic diamines. These materials are all much less soluble, and when crystalline have much higher melting points than the analogous polyesters (51).

b. Polyamines. v. Braun observed (52) that the compound $\text{NH}_2(\text{CH}_2)_6\text{Cl}$, unlike its immediate lower homologs, when it reacts with itself yields only a very small amount of the volatile cyclic base. The chief product is an undistillable, waxy solid, but this has never been studied in detail.

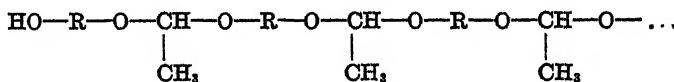
The formation of quaternary ammonium salts from various compounds of the series $(\text{CH}_2)_n\text{N}(\text{CH}_2)_n\text{Br}$ has been studied (53). The products have been assumed to be cyclic monomers or dimers,

but it still remains to be demonstrated that none of them are linear polymers.

c. *Polyacetals*. The reaction between glycols and acetaldehyde (or acetylene) presents the possibility of forming cyclic acetals,



or polyacetals



This reaction has been studied by Hill and Hibbert (8). Ethylene and trimethylene glycols gave in excellent yields the cyclic acetals which are five- and six-atom rings. Tetramethylene glycol gave in poor yield a volatile compound which was apparently the monomeric cyclic acetal containing a seven-atom ring. A considerable part of the product was an undistillable sirup. The products from octamethylene and decamethylene glycols were also undistillable sirups. No molecular weight determinations are recorded, but it may be assumed that the undistillable products are polymeric. An analogous compound prepared in this laboratory (51) from benzaldehyde and diethylene glycol had an apparent molecular weight of about 1370.

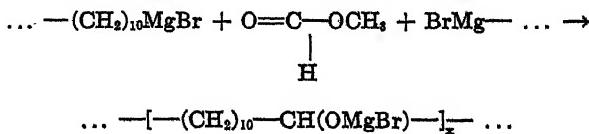
d. *Polyanhydrides*. Dibasic acids of the series $\text{HOOC}(\text{CH}_2)_n\text{COOH}$ are readily converted into the corresponding anhydrides. Malonic anhydride, the first member of the series, is polymeric (54). The next two members, succinic and glutaric anhydrides, are known only as the monomeric five- and six-membered rings. The anhydrides of all the higher acids are polymeric.¹⁴ The

¹⁴ The known examples are anhydrides of adipic, pimelic, suberic, azelaic, sebatic (55) and hexadecamethylene dicarboxylic (51) acids. These if monomeric would be respectively seven-, eight-, nine-, ten-, eleven-, and nineteen-membered rings. Adipic anhydride can be obtained in both a monomeric and a polymeric form (56).

monomeric anhydrides are macrocrystalline, readily distillable solids; the polymeric anhydrides are non-volatile microcrystalline powders or waxes, and they are less soluble than the monomeric anhydrides. Both types are very reactive, but qualitatively their chemical behavior is not the same. The monomers react with aniline to give pure monoanilide; the polymers give a mixture of acid, monoanilide, and dianilide in the ratio 1:2:1. This is precisely in accordance with the calculated behavior of a very long chain having the general structure.



e. Grignard reactions. Bifunctional Grignard reagents such as $\text{BrMg}(\text{CH}_2)_5\text{MgBr}$ are capable of reacting with bifunctional reactants (dialdehydes, diketones, simple esters, etc.). The products may be rings or long chains. Several five- and six-atom rings have been prepared in this way, and in poor yield one seven-atom ring (57). In this laboratory it has been found (51) that decamethylene dimagnesium bromide reacts readily with methyl formate:



The final product is a microcrystalline solid readily soluble in various organic solvents and melting at about 120°C . Its chemical and analytical behavior shows that it is the expected linear polyalcohol containing about eight of the structural units $—(\text{CH}_2)_{10}—\text{CHOH}—$.

In the formation of bifunctional Grignard reagents some coupling always occurs: dibromides of the formula $\text{Br}(\text{CH}_2)_n\text{Br}$ yield considerable amounts of $\text{BrMg}(\text{CH}_2)_{2n}\text{MgBr}$ and progressively smaller amounts of higher coupling products. It is a curious fact that this reaction does not occur intramolecularly even in the case of pentamethylene bromide where it would lead to the formation of a five-membered ring (154).

The coupling of simple Grignard reagents can be effected by the

action of iodine, and this method has been applied by Grignard and Tcheoufaki to acetylene dimagnesium bromide (155). Products having the following formulas were isolated:



and also a form of carbon, which probably resulted from a continuation of the initial reaction in the same sense.

The attempt to prepare a Grignard reagent from *p*-xylylene bromide, $\text{BrCH}_2\text{C}_6\text{H}_4\text{CH}_2\text{Br}$, leads (51) to an insoluble hydrocarbon having the composition



The value of x is probably very large.

f. Sulfur and selenium compounds. The action of sodium sulfide on alkylene halides of the formula $\text{X}(\text{CH}_2)_n\text{X}$ leads to considerable yields of the expected cyclic products $(\text{CH}_2)_n > \text{S}$ only when these are five- or six-membered rings (162). Ethylene bromide gives white amorphous insoluble products (161); when prepared under certain conditions these are capable of being depolymerized by heat to yield the cyclic dimer, diethylene disulfide. Monomeric ethylene sulfide has been prepared by Delepine (86) by treating ethylene thiocyanate with sodium sulfide. It polymerizes spontaneously on standing. The chief product of the action of sodium sulfide on trimethylene halides is an amorphous polymer, although a small amount of the cyclic trimethylene sulfide can be obtained under certain conditions (87). Hexamethylene iodide also yields an amorphous polymer as the chief product (163, 87). v. Braun suggests that its formula is probably

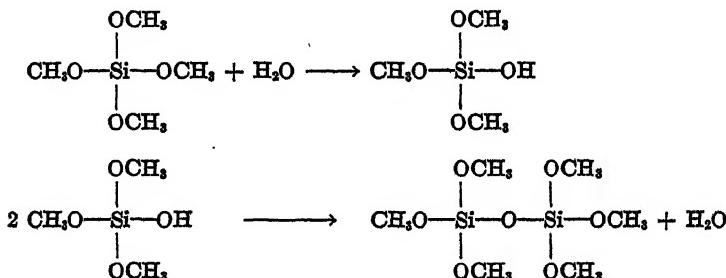


but no estimates of its molecular weight have been presented.

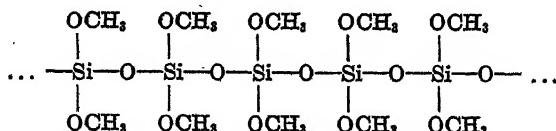
The amorphous material formed from ethylene chloride and sodium sulfide is capable of being molded into a product which is very resistant to solvents and somewhat resembles rubber in its properties. One may infer that its molecular weight is very high. In recent patents (159) it is claimed that similar products can be obtained from alkylene halides generally.

Morgan and Burstall (160) state that the interaction of sodium selenide and the requisite alkylene dibromide leads readily to the production in good yields of cyclic seleno hydrocarbons containing five- or six-membered rings, but trimethylene bromide and sodium selenide give little cycloselenopropane. The main product is a sixfold polymer melting at 38–40°C.

g. Miscellaneous. A curious spontaneous progressive coupling is found in the action of water on methyl orthosilicate. The reaction may be formulated as follows:



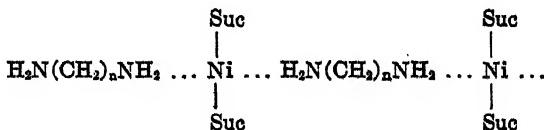
Partial hydrolysis and coupling of this dimeric product lead to a tetramer, and so on. By suitably adjusting the initial ratio of water to ester it is possible to obtain samples representing various degrees of polymerization. The lower members have been isolated as chemical individuals by fractional distillation (156). The intermediate members are viscous undistillable liquids soluble in organic solvents. Solutions of these polymers are used in the preparation of paints having unusual properties (60). Films prepared from these paints harden by a continuation of the initial progressive hydrolysis and coupling, which yields as the final product pure silica. This synthesis incidentally furnishes an elegant proof of the three-dimensional polymeric structure of silica. The OCH_3 groups are responsible for the coupling, which results in the formation of long chains from the monomeric ester.



Since each silicon atom in these chains still bears two OCH_3 groups, the coupling can continue in the other dimensions, yielding finally a three-dimensional lattice in which each silicon atom is joined (through oxygen) to four others.

Metallic sodium acts on compounds of the type R_2SiCl_2 yielding chain structures composed of $-\text{SiR}_2-\text{SiR}_2-$ groups (157). These products are for the most part amorphous and glue-like.

The specially pronounced tendency toward the formation of five- and six-membered rings is frequently manifested in complex coördination compounds (148) and complex formation frequently fails when it would involve the formation of larger rings. Ethylene and trimethylene diamine yield crystalline nickelotriene succinimide compounds of the formula $[\text{Nien}_3]\text{Suc}_2$ and $[\text{Nitr}_3]\text{Suc}_2$, but the compounds from tetra- and penta-methylene diamines are amorphous and, according to Tschugaeff (114), they must be represented as chains of unknown length:



Bifunctional couplings probably occur in many reactions of oxidation and reduction. Busch and Schmidt have shown (22) that the catalytic reduction of aryl halides under some conditions proceeds to a considerable extent as follows:



When the same reaction is applied to *p*-diiodobenzene, terphenyl, $\text{C}_6\text{H}_5\cdot\text{C}_6\text{H}_4\cdot\text{C}_6\text{H}_5$, and *p*, *p'*-diphenyldiphenyl are obtained.

A preliminary stage in the oxidation of aniline to aniline black is the formation of long chains of the following type (20),



In a similar way the oxidation of phenols may lead to the formation of polyphenylene ethers (21). Substituted polyphenylene ethers of high molecular weight are obtained by the condensation polymerization of silver salts of halogenated phenols (173).

4. Stereochemical factors involved in condensation polymerization

Bifunctional reactions always present the possibility of following two courses—they may lead to ring closure, or to progressive coupling—but the very numerous studies of such reactions have been concerned almost exclusively with ring closure. The possibility of progressive coupling has frequently been ignored or rejected as unlikely, and highly improbable cyclic structures have occasionally been assigned to products on the ground merely that they were the result of bifunctional reactions. In other cases the actual products have been discarded simply because they were obviously not the expected cyclic products. This attitude has certainly been partly responsible for the comparative meagerness of the literature on condensation polymers. The examples cited in the previous paragraphs show, however, that bifunctional reactions may proceed in a strictly normal fashion by progressive coupling and result in the formation of large molecules, and that, considering any particular homologous series, this type of reaction is the rule and ring closure is the exception.

This is not an especially astonishing conclusion. Intermolecular reaction is a perfectly general phenomenon; intramolecular reaction is a peculiar and special kind of happening. This is immediately evident from the consideration that if two groups are to react they must meet, i.e., they must approach each other very closely. If the groups are not present in the same molecules, such approach is always possible as long as the molecules are free to move about. But two groups, x and y , that are present at opposite ends of a single molecule are capable of approaching each other only if the architecture of the molecule permits, and even if such approach is permissible it does not necessarily follow that it will occur, or if it occurs that it will be effective. Meanwhile the x groups of this molecule are continually colliding with y groups of other similar molecules. From this standpoint progressive coupling in bifunctional reactions is inherently more probable, generally speaking, than ring closure, and the latter, if it occurs to the exclusion of the former, must be peculiarly favored by some special factors.

The reasons for the great difference in the relative ease with

which different types of rings are formed has been the subject of much speculation, and two factors that have frequently been assumed to be of self-evident importance are the relative energy content of the rings and their stability or degree of strain. There is, however, no theoretical justification for this assumption (58), and the facts show that neither of these factors can possibly be decisive. Thus, the action of sodium on propylene bromide yields cyclopropane, not cyclohexane, although the latter has a much lower energy content per unit and is less highly strained than the former.

On the other hand spatial relations in the reacting compound must of necessity have a great influence on the possibility of ring formation. The atoms of the benzene ring, for example, and all the atoms joined directly to it lie in the same plane (59), and to link the two *para* positions of the ring together through a chain of less than four or five accessory atoms would require a very improbable degree of distortion. Many attempts have been made to bring about such linkings, but the supposedly successful examples have proved on reexamination to be fictitious (62). The same thing is true (61) of alleged examples of ring closure through the *p* and *p'* positions of diphenyl.

The stereochemistry of simple aliphatic chains is a rather complicated matter. Ruzicka's discovery that large aliphatic rings are no less stable than small ones is impossible to reconcile with the Baeyer strain theory, and the latter has been superseded by the Sachse-Mohr theory (66) which permits the existence of large rings in non-planar and strainless forms and is supported by a great deal of other evidence besides. The essential assumption in the Sachse-Mohr theory is simply the usual one that the four valences of the carbon atom are directed toward the corners of a tetrahedron and that there is free rotation about each single bond in a chain. Space models embodying these features can easily be made by joining small wire tetrahedra with rubber tubing in such a way that the arms that are being connected overlap. Rings constructed with such models show graphically that a five-atom ring is uniplanar and free from strain; a six-atom ring is highly strained unless two of the atoms are allowed to lie

in a different plane from the other four; larger rings if they are to exist must be multiplanar and strainless, and they possess a mobility which makes it possible for them to assume a great multiplicity of shapes.¹⁵

The variety of possible configurations of an open chain is much greater than in a ring and it increases rapidly with the length of the chain. In the crystals of fatty acids and paraffins the chains probably are rigidly extended into a zigzag structure (13); but a model of such a chain is exceedingly flexible and mobile, and it seems very improbable that in the liquid condition or in solution the molecules retain their rigidly extended form. No doubt certain possible configurations are more probable than others, but there is at present no means of knowing just what these are.¹⁶ It is clear, however, that the relative probability of close approach of the ends of a chain diminishes very rapidly as the length of the chain increases. Owing to the fewness of their separate points of rotation, five- and six-atom chains can assume relatively few configurations. In fact, if a space model of such a chain is supported at one of the bonds the entire structure can be rotated in such a way that the freely moving and unsupported ends collide at each rotation. This shows that there is a certain inevitability in the closure of such chains. Longer chains on the other hand can assume an extraordinary multiplicity of shapes without any close approach of the ends. The ends of the model can be brought together arbitrarily without any resistance, but as Mohr has pointed out (66) in the molecule itself one is dependent upon

¹⁵ Apparently the shape that they actually tend to assume involves the close approach of opposite sides of the ring; the ring consists essentially of two parallel chains joined at the end (67, 144, 145).

¹⁶ Attempts to decide this question by studies of x-ray diffraction (88), electric moment (63), ionization constant (65), and numerous other properties (143) have led to the following rather contradictory conclusions: (a) the chains are straight zigzags; (b) they are straight zigzags except for the five-atom chain which is coiled; (c) they have a helicoidal configuration which brings the first and fifth atoms very close together and introduces an anomaly in properties when the length of the chain is five, ten, or fifteen atoms. None of these configurations provides any mechanism for the close intramolecular approach of the ends of chains longer than six atoms.

random collision,¹⁷ "which will bring about a given form the more rarely the more forms are possible, i.e., the longer the chain."

One obvious and important implication of this theory is that a cyclic structure for linear high polymers is very improbable. The formation of such polymers usually depends upon the absence of any tendency toward ring closure in the early stages of the coupling, and the probability of ring closure will diminish as the length of the chain increases. This implication is also in accord with the facts. It is true that cyclic formulas have been assigned to various high polymers by Staudinger and by other investigators, but none of these formulas has been established.¹⁸ On the other hand, in a variety of instances open chain structures have been experimentally proved.

The presence of substituents and the nature of the terminal groups may be expected to modify the stereochemical behavior of chains. Thus, if two atoms of a chain are adjacent atoms of a benzene ring their position with respect to each other is fixed, and the chances of intramolecular reaction are greater than in an analogous simple chain of the same length. (The latter will have

¹⁷ It seems quite possible that this dependence upon random collision is not inherently necessary. It may be that some selective control over molecular form is possible by the use of the orienting effects at surfaces, and such factors may perhaps come into play in the synthesis of large rings in nature. This suggests the possibility of some very interesting studies of a novel kind in surface chemistry.

¹⁸ In this connection merely negative evidence, e.g., the failure to detect terminal groups, is worthless. Terminal groups may become lost or mutilated, or they may be present and yet fail to react. High polymers are frequently very sluggish in their chemical behavior.

The tendency to resort to cyclic formulas does not aid in clarifying the problems of high polymers. One can assume that the rubber molecule is an enormously large ring, but rubber is very susceptible to degradation by oxygen, heat, mechanical action, etc., and the first step in such degradation must cause the ring to be ruptured. This product then presents all those problems which the assumption of a cyclic structure was designed to evade.

It seems evident that all polymerizations, whatever their mechanisms, must be progressive stepwise reactions. The simultaneous combination of 100 molecules presents insuperable kinetic difficulties. If this view is correct, ring closure can occur only as the last step in the reaction chain. But at this stage the ends of the chain must be more remote from each other than at any earlier stage, and the opportunities for ring closure must be at a minimum.

one more axis of rotation than the former.) It is not surprising therefore that the majority of the known seven- and eight-membered rings have at least two of their atoms members of a benzene ring. The compound $\text{NH}_2(\text{CH}_2)_6\text{Cl}$ reacts with itself intramolecularly only to a slight extent, while with $o\text{-NH}_2\text{C}_6\text{H}_4(\text{CH}_2)_4\text{Cl}$ ring closure is almost quantitative (68). Other similar examples might be cited (50, 163).

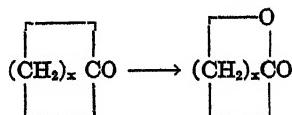
There is also some evidence to indicate that even simple substituents such as methyl groups on a chain may increase the tendency toward the formation of larger rings. Researches in the diphenyl series (69) have established that substituent groups suitably placed may completely inhibit rotation about a nearby single bond, and a similar effect in aliphatic chains is at least conceivable. Almost any restriction of the freedom of rotation of the atoms of a chain would, on the basis of the Sachse-Mohr theory, increase the chances of ring formation.

The influence of the nature of the terminal groups is seen in the fact that ω -hydroxy acids on dehydration give both lactone and polyester, while attempts to prepare seven-atom cyclic esters from dibasic acids and dihydric alcohols yield only polyesters. Dilution also may be expected to favor ring formation as compared with progressive coupling (70), but all these effects are comparatively small.

a. *Large rings.* The question arises whether large rings are ever formed as the normal primary products of bifunctional reactions. Meyer and Jacobson in their textbook (147) devote over fifteen hundred pages to five- and six-membered heterocyclic compounds and only seventeen pages to all larger rings. Most of the examples of this class of compounds that they accept as authentic fall into one or more of the following types: the ring contains only seven or eight atoms; two or more atoms of the ring are members of a benzene nucleus; the yields are very poor; the compound is formed by ring widening. There remain a few examples (e.g., the cyclic duplomercaptal derived from acetone and pentamethylene dimercaptan) in which the evidence for a very large cyclic structure is good; but this evidence is no better than that on which the sixteen-membered cyclic formula for

ethylene succinate was based (41), and ethylene succinate when reexamined (32, 34) proved to be a linear polymer of high molecular weight.

Concerning the identity of large rings described by Ruzicka, it is not possible to entertain any doubt at all, but the methods required for the preparation of these materials present some illuminating peculiarities. His lactones were obtained from the corresponding cyclic ketones by oxidation (25),



i.e., the ring was already established in the starting material. All attempts to prepare the lactones by a bifunctional reaction, e.g., from the hydroxy acids or from the silver salts of the bromo acids were unsuccessful. The ketones themselves (71) were obtained by heating the thorium salts of the dibasic acids to a very high temperature (400–500°C.). These conditions of violence lead to thermal rupture, and, whatever the primary products may be, the ultimate products must, for the most part, be volatile materials of fairly low molecular weight. The cyclic ketones are found mixed with a great variety of other materials among these volatile products. It is at least conceivable that the primary products in this reaction are linear polyketones and that the cyclic ketones are thermolysis products of the polymer.

In this connection it is interesting that large heterocyclic rings can also be obtained by thermolysis of the polyesters. Thus when polyethylene succinate is heated to 300°C. in a vacuum, it decomposes and yields a carbonaceous residue and a gaseous and liquid distillate. From the latter a small amount of the sixteen-membered cyclic dimer can be obtained. Similar cyclic products have been obtained from tetramethylene carbonate, from trimethylene oxalate and from the potassium salt of the acetyl derivative of hydroxydecanoic acid. The anhydride of sebacic acid can also be depolymerized under certain conditions to a cyclic dimer. The properties of these compounds are listed in

TABLE I
Large cyclic esters and anhydrides

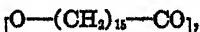
COMPOUND	FORMULA	ATOMS IN RING	MELTING POINT °C.	REFERENCES
Ethylen succinate.....	$[O(CH_2)_2OCO(CH_2)_2CO—O(CH_2)_2OCO(CH_2)_2CO]$	16	130	42, 34
Tetramethylene carbonate....	$[O(CH_2)_4OCO—O(CH_2)_4OCO]$	14	173	33
Trimethylene oxalate.....	$[O(CH_2)_3OCOCO—O(CH_2)_3OCOCO]$	14	176	64, 35
Dimeric lactone of ω -hydroxy-decanoic acid.....	$O(CH_2)_6CO—O(CH_2)_6CO$	22	95.5	30
Dimeric sebacic anhydride....	$OCO(CH_2)_8CO—OCO(CH_2)_8CO$	22	68	51

table 3. Their identity is established by analytical data and repeated molecular weight determinations. They are distinguished from the corresponding polymers by their definite macrocrystallinity and relatively great solubility. The attempts to obtain corresponding monomers which would be seven-, eight-, and eleven-membered rings have been unsuccessful. It seems possible that this failure may be due to purely practical difficulties, but so far as the data go they are in agreement with the fact that Ruzicka found the yields of the cyclic ketones of eight to twelve atoms to be much less than the yields of the larger rings. This fact is not easy to explain on the basis of the simple Sachse-Mohr theory presented above. A possible explanation has recently been offered by Stoll and Stoll (67).

III. POLYMERIZATION INVOLVING CYCLIC COMPOUNDS

1. *Six-membered cyclic esters*

Among cyclic esters the property of undergoing reversible polymerization is characteristic of and peculiar to the six-membered rings. γ -Butyrolactone cannot be polymerized, and no corresponding polyester is known. Higher lactones, e.g.,



are stable substances which show no tendency to polymerize spontaneously, although polymers are the only products of the self-esterification of the corresponding hydroxy acids. On the other hand, δ -valerolactone, a mobile liquid, gradually changes on standing to an opaque solid polymer, and from this the lactone can be regenerated by heating.

Various isolated examples of the polymerization of the six-membered esters have been reported, but only a few of them have been described in any detail. In this laboratory some study (mostly unpublished) has been made of the mechanism of this phenomenon.

The esters now known to exhibit this behavior are listed in table 4. (In most cases neither the molecular weights nor the melting points of the polymers can be regarded as very significant,

TABLE 4
Six-membered cyclic esters and their polymers

MONOMER	FORMULA	MONOMER		POLYMER		REFERENCES
		Melting point °C.	Molecular weight	Melting point °C.	Molecular weight	
δ -Valerolactone.....	$[O(CH_2)_4CO]$	Liquid	114	52-53	2180	72, 51
α -Propyl- δ -valerolactone.....	$[O-(CH_2)_3CH(CH_3)CO]$	Liquid	142	Syrup	1000	51
Lactone of hydroxyethylglycolic acid.....	$[O(CH_2)_2OCH_2CO]$	31	102	62-64, 87-89	460, 1700	73, 51
2,3,4-Trimethyl-arabonolactone.....	$[OCH_2[CH(OCH_3)]_3CO]$	45	170	135-138	2000	74
Ethylen oxide.....	$[O(CH_2)_2OCOCO]$	143	116	159, 172	2300, insoluble	35
Propylene oxalate.....	$[OCH_2CH(CH_3)OCOCO]$	142	130	176-180	Insoluble	35
Trimethylene carbonate.....	$[O(CH_2)_3OCO]$	47	102	Glassy	4000	33
Glycolide.....	$[OCH_2CO-OCH_2CO]$	86	116	223	Insoluble	75, 76
Lactide.....	$[OCH(CH_3)CO-OCH(CH_3)CO]$	125	144	Resin	3000	77, 51

since both are dependent upon the conditions under which the polyester is formed.)

Some of these examples present special features worthy of mention. Monomeric trimethylene carbonate is a very soluble, crystalline solid. If it is heated with a trace of potassium carbonate to 130°C. for a few minutes, the mobile melt suddenly becomes very viscous and evolves a small amount of gas. The colorless sirup on being cooled solidifies to a stiff mass which shows an apparent molecular weight of about 4000. When heated in a vacuum this mass distils almost quantitatively, and the distillate consists of pure monomer.

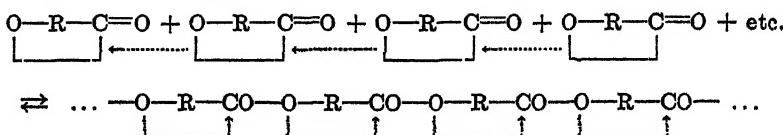
Ethylene oxalate is a solid crystallizing in transparent, flat diamonds, melting at 143°C. These on standing for a few days in a stoppered container disintegrate to a powder, which consists of a mixture of polymers. By extraction with cold solvents it can be separated into two definite fractions, one melting at 159°C. and having an apparent molecular weight of about 3000, and one melting at 173°C. and having an unknown but probably much higher molecular weight. These fractions on standing for a few days lose their identity; they are partly converted into each other and into monomer. It is interesting to note in connection with the rapidity of these transformations that monomeric ethylene oxalate is exceedingly sensitive to hydrolysis.

Drew and Haworth (74) have obtained the lactone of 2,3,4-trimethyl-*l*-arabonic acid in crystalline form (m.p. 45°C.) and have observed that in the presence of traces of hydrogen chloride it is converted into a crystalline polymeric powder. This has a considerably higher melting point, a lower solubility, and a lower specific rotation than the lactone. Its molecular weight (about 2000) indicates that it is derived from about ten molecules of the latter. At 175°C. it distils completely *in vacuo*, and the distillate consists of pure lactone. Drew and Haworth ascribed a linear polyester structure to this polymer and were inclined to accept its crystallinity as evidence of its absolute homogeneity, but it seems much more probable that it is a polymeric mixture.

The ease of polymerization of the six-membered cyclic esters appears to be related to their susceptibility to hydrolysis. In

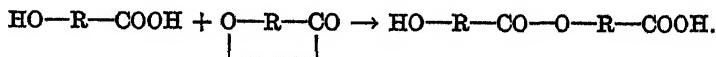
general, substitution increases the resistance to hydrolysis and diminishes the tendency to polymerize. Thus glycolide polymerizes spontaneously at the ordinary temperature, but lactide only on being heated or exposed to the action of catalysts. Attempts (51) to bring about the polymerization of analogs of glycolide derived from some of the higher α -hydroxy fatty acids have been unsuccessful.

These polyesters are formed from the monomers by a process of ester interchange.



and the reverse transformation proceeds by a similar mechanism, as indicated by the arrows. Both transformations are catalyzed by acids and bases—typical interchange catalysts.

Direct evidence for this interchange mechanism is found by polymerizing δ -valerolactone in the presence of various amounts of chloroacetic acid (51). This acid actually participates in the reaction, and according to the amount present it regulates the length of the chains produced. The effect of the acid may be compared with that which water might produce. One molecule of water with one molecule of the lactone would simply yield the hydroxy acid. A smaller amount of water would yield some hydroxy acid and this might react with the lactone to form a dimeric ester:



If the amount of water were quite small the polyester molecule would have to be quite large. Apparently chloroacetic acid functions in precisely the same way, and a comparison of the halogen content and neutral equivalent of the polyesters produced under various conditions not only establishes the open chain structure of the polymer, but also clearly indicates the mechanism of the reaction.

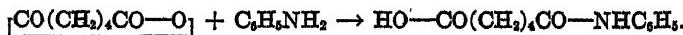
It remains to explain why only the six-membered esters are capable of undergoing reversible transformation of this kind. The presence of some strain in such esters is indicated by the great instability of δ -lactones as compared with γ -lactones (78, 79). The Sachse-Mohr theory permits the existence of six-atom rings in two isomeric strainless forms; but in simple rings these two isomeric forms have never been realized, and one is forced to conclude either that such rings are uniplanar and hence highly strained, or that the two isomeric forms are in dynamic equilibrium. Practically this amounts to the same thing, since at each conversion the molecule must pass through the uniplanar position of strain. These strains can be relieved by an ester interchange resulting in the formation of the polyester. The easy depolymerization of the resulting polyester is readily explained by the high degree of probability of the close approach of points six atoms apart in a chain. Polyesters whose structural units are longer than six atoms are not readily depolymerized because of the improbability of close approach of the requisite atoms of the chain. Cyclic esters of five atoms or of more than six atoms are not polymerized because their cyclic systems are free from strain.

In the formation of six-membered cyclic esters from open chain compounds, either the monomer or the polymer may first be isolated according to the experimental conditions, but in either event it is not easy to prove that the form isolated is the primary product and not a polymerization or depolymerization product of the primary product. It seems fairly certain that simple δ -lactones may be formed directly from the corresponding hydroxy acids. On the other hand there is evidence (19) that the self-esterification of lactic acid yields only polylactyllactic acids and that lactide, when it is formed, is produced from these by depolymerization. Since lactide can itself be polymerized to a poly-lactyllactic acid, the latter furnishes an example of a polymer that can be formed either by addition or by condensation polymerization. In most cases, no doubt, either the cyclic ester or the polymer can be formed first depending upon the conditions.

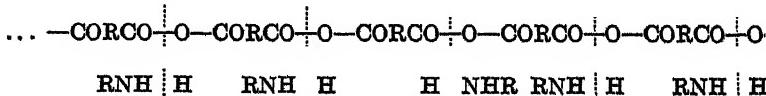
2. Adipic anhydride

The behavior of adipic anhydride (56) illustrates the fact that the property of undergoing reversible polymerization of this type is not peculiar to the polyesters. In this case it is the seven-atom ring that is unstable. Succinic and glutaric anhydrides, the five- and six-atom rings, cannot be induced to polymerize. Sebacic anhydride is polymeric and cannot be depolymerized to a monomer. Adipic anhydride, when it is prepared from the acid, is also polymeric. It is a waxy solid which separates from solvents as a microcrystalline powder. On being heated *in vacuo* it is depolymerized to a considerable extent, and distillation occurs. The distillate is the monomeric adipic anhydride, a liquid freezing at about 21°C. In the presence of traces of moisture this very rapidly reverts to the waxy polymeric form.

A direct chemical proof of the actual structural difference between the two forms of adipic anhydride is possible (56). Both forms react very rapidly with aniline. The monomer, since it contains in its molecule only a single anhydride linkage, can yield only the monoanilide and this is in fact the only product formed.



But with the polyanhydride the nature of the final monomeric products will depend upon which side of the anhydride linkage is involved at successive steps:

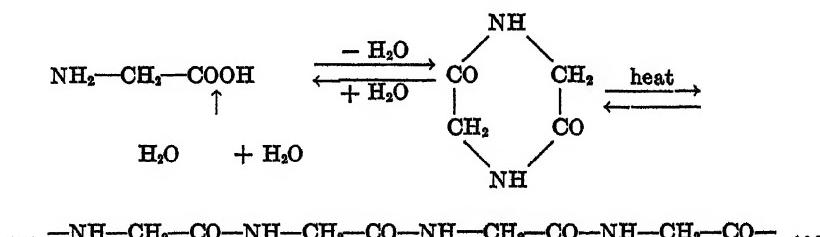


If the molecule is infinitely long, i.e., if it is so long that the terminal groups can be ignored, considerations of probability indicate that the product will be 50 per cent monoanilide, 25 per cent dianilide, and 25 per cent acid. Within the experimental error these are the yields actually obtained. This indicates that the molecule must be made up of at least ten or fifteen structural units. A cyclic polymer would of course give the same result, but

a low molecular weight is excluded by the properties of the material, and a cyclic structure of any kind is made improbable by considerations already discussed.

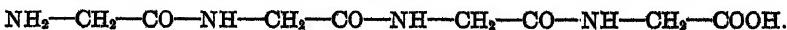
3. Diketopiperazines and polypeptides

The curious behavior of the α -hydroxy acids is especially interesting in connection with the structure and formation of proteins, since these are for the most part derived from α -amino acids. If glycine were to behave like glycolic acid, the following transformations could be realized.



It appears that diketopiperazine can be polymerized, though with some difficulty (83), and polypeptides are readily obtained by the self-condensation of glycine and its esters.

Balbiano (80) in 1900 showed that when glycine is heated in glycerol solution it loses water and yields as the principal product a horn-like mass, together with a small amount of diketopiperazine. The horn-like material is practically insoluble in all solvents except hot concentrated hydrochloric acid, and by hydrolysis it is converted to glycine. Later this reaction was studied in more detail by Maillard (81), who showed that according to the conditions and time of heating more or less of the dimeric anhydride or the horn-like polymer can be obtained. He also isolated an intermediate individual, triglycylglycine,



This polypeptide is soluble in water, and Maillard made the curious observation that if its aqueous solutions contain diketopiperazine they deposit on standing an insoluble material. Analytical evidence indicated that this material is a hexapeptide.

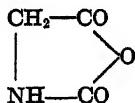
Except for the solubility effect in aqueous solutions there is no obvious reason why this coupling of the diketopiperazine with the polypeptide should stop at the hexapeptide stage. In glycerol solution one may suppose that it continues progressively and results in the building up of a very long chain. The final horn-like product would then be a polypeptide containing a very large number of structural units—perhaps forty or more. Maillard assigns to this horn-like polymer the structure of a cyclopolycyclglycylglycine, but no convincing proof of the cyclic formula is presented, and on general grounds an open chain formula seems more probable.

The production of glycine anhydrides in glycerol solution probably involves the formation of glycercyl esters as transitory intermediates, but diketopiperazines and polypeptides can also be obtained by heating amino acids in the absence of a solvent. Curtius and Benrath (82) state that at high temperature the chief product from glycine is a pentapeptide.

The esters of glycine couple with themselves much more readily than the free acid. Curtius (84) showed that glycine ethyl ester loses alcohol even at the ordinary temperature. When water is present the chief product is diketopiperazine, but some polypeptide ester is formed at the same time. Under anhydrous conditions the latter is the chief product. When glycine ethyl ester is dissolved in a little dry ether and allowed to stand at the ordinary temperature, a crystalline precipitate gradually accumulates. This consists almost exclusively of the ethyl ester of triglycylglycine. The esters of such polypeptides are of course capable of coupling with themselves to form still longer molecules. Fischer (85) observed that when the methyl ester of alanylglucylglycine is heated to 100°C. it yields some hexapeptide ester and some less soluble amorphous material. Curtius (84) found that when his triglycylglycine ester was heated to 100°C. *in vacuo* it was converted into an insoluble infusible material having the composition —NHCH₂CO—. He assigned to this material the structure of a cyclic octapeptide, but a much more highly polymeric open chain structure seems more probable.

In connection with the polypeptides the behavior of the an-

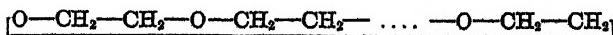
hydrides of the N-carboxy amino acids may be mentioned (89). The compound



dissolves readily in water at 0°C. At 15°C. carbon dioxide is evolved and an aqueous solution of glycine results. If, however, the compound is rubbed with a little water at room temperature or is heated, carbon dioxide is lost immediately and an insoluble infusible material is the only other product. This has the composition —NHCH₂CO— and on hydrolysis it yields glycine. It appears to be similar to the horn-like product obtained by Balbiani (80). It is quite probably a polypeptide of very high molecular weight. The anhydrides of other N-carboxy α -amino acids behave in a similar fashion (90).

4. Ethylene oxide (14, 15)

Ethylene oxide provides another example of the polymerization of a cyclic compound not involving double bond unsaturation. The polymerization is induced by various catalysts such as alkali metals, tertiary amines, and stannic chloride. Ultra-violet light or Florida earth is not effective. The product is a solid readily soluble in water and in most organic solvents except ether. Its properties vary somewhat according to the method of preparation. By fractional precipitation it can be separated into fractions ranging in apparent molecular weight from about 400 to nearly 5000. The lowest member of this series is liquid and the highest one a solid melting at about 59°C. The polyethylene oxides are not depolymerized by heat; but above 300°C. they decompose and yield a complicated mixture of products containing some acetaldehyde and acrolein. Roithner (15) assigns to polyethylene oxide the following formula,



but the evidence for this structure is largely negative. Unpublished work in this laboratory has led to the detection of

terminal hydroxyl groups, indicating that the molecule is an open chain.

Ethylene oxide also polymerizes under certain conditions to yield dioxane (16). Apparently this shows no tendency to polymerize further as the six-membered cyclic esters do.

IV. ADDITION POLYMERIZATION OF UNSATURATED COMPOUNDS

This is the only type of polymerization that is recognized by the usual definitions. Thus Cohen states (91) that "the property of undergoing polymerization is peculiar to unsaturated compounds, from a natural tendency to saturate themselves." So far as the formation of materials of high molecular weight is concerned, such reactions are much less clear-cut than bifunctional condensations, for the latter involve only the application of the known reactions of typical functional groups, and the general structural plan of the product may be inferred directly from the structure of the starting materials. On the other hand, no clue to the intimate details of the mechanism of self-addition can be found in the reactions of the compound concerned with any compounds other than itself. Ethylene oxide and certain cyclic esters and anhydrides already discussed polymerize by self-addition, but these reactions in some respects are radically different from the polymerization of compounds containing multiple linkages.

Under sufficiently drastic conditions almost any compound can be converted into a material of high molecular weight. Thus methane when subjected to the action of alpha particles or to the silent electrical discharge is partly transformed with loss of hydrogen into higher hydrocarbons (165), and benzene in the electrodeless discharge is converted into insoluble, amorphous products (166). The reaction of acetylene with itself illustrates how complicated the polymerization of an unsaturated compound may be. Polymerization at elevated temperature in the presence of active charcoal leads to a complicated mixture of hydrocarbons containing considerable amounts of benzene and naphthalene (167); the use of copper- or magnesium-containing catalysts yields (168) a completely insoluble amorphous powder (cuprene) of

unknown structure, and similar products are obtained by the action of ultra-violet light (169), cathode rays (170), or alpha particles (171) on acetylene. On the other hand, the action of the silent electrical discharge (172) at low temperature leads to the formation of considerable amounts of liquid products which contain highly unsaturated open chain compounds.

The brief discussion of addition polymerization contained in the following paragraphs is confined to a few of the simplest and most thoroughly studied cases.

1. Ethylene and other olefines

The rather extensive literature on the polymerization of ethylene has recently been reviewed by Stanley (92). Ethylene polymerizes less readily than most of its homologs and derivatives. The polymerization is accelerated by heat, pressure, ultra-violet light, the silent electric discharge, and by certain catalysts such as sulfuric acid (especially in the presence of salts of copper and mercury), zinc chloride, boron trifluoride, and aluminum fluoride. The products are usually oils having a wide boiling range. In general, they are not exclusively of the C_nH_{2n} type; hydrogenation, dehydrogenation, and cyclization may occur at the same time. These products are commercially valuable, but too complicated to furnish any clue as to the mechanism of their formation.

Mignonac and Saint-Aunay (93), however, have succeeded in isolating the first products formed in the action of the silent electrical discharge on ethylene. These products are butene-1 and hexene-1. Pease (94) has found evidence of the presence of butene-1 in the products of the thermal polymerization, and from a study of the kinetics has concluded that this is a chain reaction. In effect at least this reaction involves at the first step the addition of ethylene, as $H + CH=CH_2$, to the double bond of another molecule of ethylene, and then a similar addition to butylene.

Lebedev and his collaborators (95) have presented interesting data on the early stages of the polymerization of isobutylene. They used especially Florida earth as a catalyst and obtained mixtures from which a whole series of polymers up through the

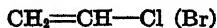
heptamers was isolated. They found that the trimer was not polymerized under the conditions that lead to higher polymers from either the monomer or the dimer. Hence the higher polymers must be built up by the successive addition of monomer or dimer. As to the precise structure of these polymers very little is known.

2. Vinyl compounds

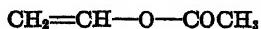
Substituted ethylenes of the type $\text{CH}_2=\text{CH}-\text{R}$, in which R is a negative group, polymerize much more readily than does ethylene itself. Examples are:



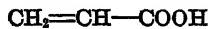
Styrene (96)



Vinyl chloride (bromide) (97)



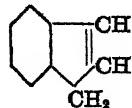
Vinyl acetate (98, 142)



Acrylic acid (99)



Acrolein (100)



Indene (101, 105)

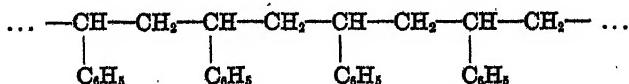
The most extensive and important studies of the polymerization of vinyl compounds are those carried out by Staudinger and his coworkers. The behavior of styrene may be taken as typical. On standing or on being heated, this mobile, volatile liquid first becomes more viscous, then changes to a more or less tough elastic jelly, and finally it may become converted into an exceedingly hard brittle mass. This change is powerfully catalyzed by light and by atmospheric oxygen, and it is inhibited by certain antioxidants such as hydroquinone. Other catalysts that are effective in accelerating the change are organic peroxides and certain metallic halides such as stannic chloride and antimony chloride. If a little stannic chloride is added to an alcohol solution of styrene, the solution becomes warm and the polystyrene quickly separates as an amorphous mass.

Polystyrene is not a definite material having a constant set of properties. By whatever method it is prepared it can be separated by fractional extraction or precipitation into fractions having the same composition but different properties and molecular weights. The lower members are readily soluble in ether and the highest members are quite insoluble. The apparent molecular weights range from 1000 up to 25,000 or more (perhaps as high as 200,000). All these fractions are soluble in benzene, and the viscosity of the solutions increases progressively with increasing molecular weight. The average molecular weight of the crude polymer depends upon the conditions of its formation. Those formed very rapidly, e.g., by the action of heat at high temperature or by the action of stannic chloride at ordinary temperature, have relatively low molecular weights. Those formed more slowly, e.g., by spontaneous polymerization at room temperature, have much higher molecular weights.

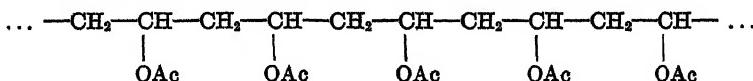
The polystyrenes having molecular weights above 10,000 show colloidal behavior; they swell before dissolving, and the viscosity of their solutions is very high. Nevertheless, there is considerable evidence to show that these solutions are true molecular dispersions (102), and that the molecule and the colloidal particle are identical.

Chemically the polystyrols are completely saturated. They do not decolorize permanganate or absorb bromine. Under sufficiently drastic conditions they can be completely hydrogenated (in the benzene nucleus) without any significant change in molecular weight (103). On being heated to about 320°C. they revert to the monomer, styrene. It is doubtful if this reversion is ever quantitative.

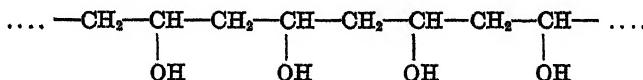
The polymerization of styrene evidently involves the disappearance of the double bond and the formation of very large molecules. The simplest and most probable structure of these large molecules is that suggested by Staudinger.



The polymerization of vinyl acetate is similar to that of styrene. In both cases the polymer first formed remains dissolved in the monomer, and the mixture of monomer and polymers can be obtained in the form of more or less tough, transparent, elastic masses. The chemical behavior of the polyvinyl acetate indicates that it is a mixture of molecules of various lengths built up according to the plan indicated in the formula

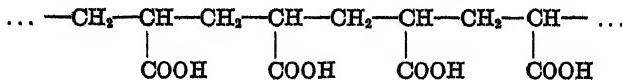


The number of molecules of monomer involved probably ranges between forty and one hundred. By fractional precipitation, samples of different average molecular weights can be obtained, and these naturally differ somewhat in their physical properties. In general the polyvinyl acetates are soluble in organic solvents but not in water. As esters they are readily hydrolyzed. The products are acetic acid and polyvinyl alcohol:



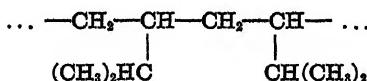
The latter as a polyhydroxy compound dissolves in water to form rather highly viscous solutions, but does not dissolve in organic solvents.

Acrylic acid also polymerizes very readily, yielding a product to which the following formula may be assigned.



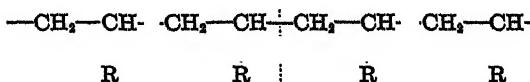
As a highly polybasic acid it forms a sodium salt which dissolves in water to yield very viscous solutions. The acid itself also dissolves in water (the most highly polymeric varieties swell very strongly and dissolve with difficulty), but does not dissolve in the typical organic solvents. On the other hand, polyacrylic esters prepared by polymerizing acrylic esters are insoluble in water, but soluble in organic solvents.

The methyl ester of polyacrylic acid on being treated with methyl magnesium iodide yields a product corresponding approximately in composition to the expected polytertiary alcohol, and this on reduction yields a hydrocarbon. Although the composition of this hydrocarbon does not exactly correspond to that required by the expected structure

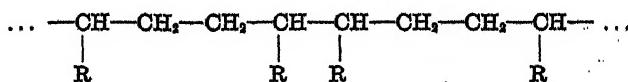


its high molecular weight proves that the ester from which it was derived is also really a material of high molecular weight, and that the units must be joined by carbon-carbon linkages.

The problem of arriving at a definite mechanism for the polymerization of vinyl compounds is complicated by the fact that neither the formation of the polymers nor their chemical behavior furnishes any certain clue to their structure. The general formula

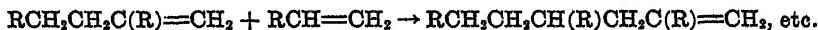


is perhaps more plausible than any other, since, as Staudinger has pointed out, it best accounts for the fact that some polyvinyl compounds (e.g., polystyrene) are smoothly depolymerized by the action of heat: the recurring substituent would weaken the linkages of the chain at the points indicated by the dotted lines. But depolymerization is never quantitative, and it is at least conceivable that a fraction of the units of each chain are arranged in the reverse fashion:



It has already been mentioned that the first step in the polymerization of ethylene is the formation of butene-1 and the second step is the formation of hexene-1. Similar terminal unsaturations have been found by Whitby and Katz in a still longer series of polyindenones, and this suggests that the most plausible mechanism

for the polymerization of vinyl compounds is "best represented as proceeding stepwise by the addition regularly of successive molecules of monomer to the double bond present at each stage of the polymerization immediately preceding" (105). This mechanism may be formulated as follows:



Staudinger disagrees with this view. He claims that the polyindenes do not have any terminal unsaturations, and from analogies based on the behavior of α -methylstyrene (104) he assigns a cyclic formula to polyindene and polystyrene. α -Methylstyrene, $\text{CH}_2=\text{C}(\text{CH}_3)\text{C}_6\text{H}_5$, polymerizes much less readily than styrene, but the reaction may be caused to occur quite rapidly by certain catalysts, e.g., stannic chloride (104). The principal product is the saturated dimer, a substituted cyclobutane. A smaller amount of a saturated trimer is formed at the same time, and in progressively smaller amounts saturated tetramer, pentamer, decamer, hexamer, heptamer, and octamer. On the basis of their physical and chemical properties Staudinger assumes that these higher polymers are respectively six-, eight-, ten-, twelve-, fourteen-, and sixteen-membered rings.

It should be observed however that these products are quite different from those obtained from styrene under similar conditions; moreover, the absence of the α -hydrogen makes it impossible for α -methylstyrene to polymerize by the mechanism suggested above for vinyl compounds generally. It is therefore scarcely permissible to conclude that the high polymers from styrene must be cyclic because the low polymers from α -methylstyrene are cyclic. Other more general grounds for rejecting cyclic formulas for linear high polymers have been presented in previous paragraphs.

The polymerization of vinyl compounds is enormously susceptible to catalytic and anticatalytic effects. Heat also accelerates the polymerization, and in general the more rapidly the polymer is formed, the lower is the average molecular weight of the

product. Oxygen and peroxides are catalysts, and antioxidants act as inhibitors. Light, especially the shorter wave lengths of the visible spectrum, accelerates the polymerization. In certain cases the presence of oxygen inhibits this photochemical effect. With the aid of certain specific catalysts, it is possible to convert styrene and certain other vinyl compounds into dimers, but these are stable substances that show no tendency to polymerize further, and they differ structurally from the dimers that have been hypothesized as intermediates in the formation of the high polymers. Under conditions that result in the formation of the latter, no polymers of very low molecular weight can be detected. All these facts indicate that the formation of the high polymer is a chain reaction. The collision of an activated molecule of monomer with another molecule of monomer yields an active dimer capable of coupling with another molecule of monomer, and the activating energy persists in the polymeric chain until it has been built up to a considerable length. Kinetic studies of the polymerization of vinyl acetate (106) and styrene (107) support the idea of a chain mechanism.

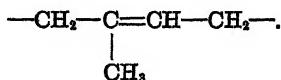
3. Dienes

Butadiene and isoprene are of especial interest in connection with rubber. Nobody knows whether in nature rubber is actually formed from isoprene or not; but it is true that rubber yields some isoprene on thermal decomposition, and that isoprene can be polymerized to a product which more or less resembles natural rubber. Similar products can also be produced from butadiene and from some of its derivatives. Efforts to produce synthetic rubber have led to hundreds of patents and various other publications. This subject is reviewed in a book by Schotz (108). There is space here to mention only two or three points.

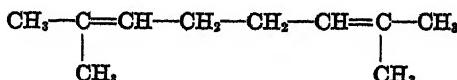
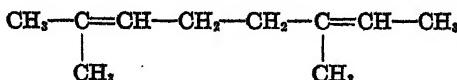
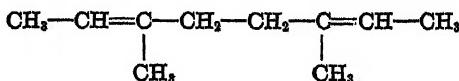
Butadiene and isoprene polymerize much less readily than styrene, vinyl acetate, etc., but apparently they are subject to the same kind of accelerating influences. Among the catalysts that have been used are oxygen, peroxides, ozonides, alkali metals, alkali alkyls. When emulsified, especially in the presence of oxygen, they polymerize more rapidly than otherwise.

It is claimed in many patents that the presence of proteins, gums, etc., in such emulsions has a favorable effect on the course of the reaction and results in products more nearly resembling natural rubber, but it seems probable that the advantages of such additions are largely imaginary. It is possible to prepare from isoprene and butadiene high polymers that have very little resemblance to natural rubber, and the problem of preparing a synthetic rubber of good quality is enormously complicated and difficult.

The structural unit in the polymeric chain from isoprene appears to be



In effect, the polymerization involves the union of a large number of these radicals derived from isoprene by the rearrangement of its bonds. Two such radicals might unite in three ways: 1,1; 1,4; 4,4. Midgley and Henne (109) have captured the first step in the reaction by carrying out the polymerization in the presence of sodium and alcohol so that the terminal valences of the dimer are hydrogenated. The structures of the three products, whose formulas are shown below, prove that all three types of combination occur.



This result indicates that the arrangement of the units in synthetic rubber formed under these conditions is less regular than in natural rubber, but it by no means proves that the actual mecha-

nism of the reaction consists in the direct union of radicals corresponding in formula with the structural unit. The 1,4 addition of $\text{H}^- + \text{---CH}=\text{C(CH}_3\text{)}\text{CH}=\text{CH}_2$ to isoprene, for example, would lead to the same result.

4. Aldehydes

The polymerization of formaldehyde under various conditions leads to polyoxymethylenes. These are microcrystalline powders, which in general cannot be melted or dissolved in organic solvents without decomposition. They have been very elaborately studied by Staudinger and his coworkers (110). These studies have proved that the molecules of the polyoxymethylenes are long chains of the type



The chains contain from forty to at least one hundred structural units. The different varieties of polyoxymethylenes are distinguished by the nature of the terminal groups: in the α -variety the terminal groups are OH; in the β -variety they are OCH_3 , and this variety is more inert chemically than the α -variety. By the action of acetic anhydride, the polyoxymethylenes are simultaneously degraded and acetylated. The product consists of a mixture of compounds of the series $\text{CH}_3\text{CO}-(\text{O}-\text{CH}_2)_x-\text{O}-\text{COCH}_3$. By distillation and crystallization, each individual of this series from $x = 1$ to $x = 20$ has been isolated in a fairly pure state. The melting points and boiling points of these individuals increase continuously with increasing molecular weight and their solubilities diminish. The successive members above $x = 20$ resemble each other so closely and their solubilities are so low, that they cannot be separated into pure fractions.

Formaldehyde also polymerizes to yield the cyclic trimer, trioxymethylene, and analogous trimers are the most common forms of the polymers of other aldehydes. It seems possible, however, that some of the metaldehydes are linear polymers of high molecular weight analogous to the polyoxymethylenes. Conant (111) has obtained from butyraldehyde by the action of very high pressure a solid polymer for which he suggests such a struc-

ture. This polymer is apparently stable only under pressure. Under the ordinary conditions it rapidly reverts to the monomer.

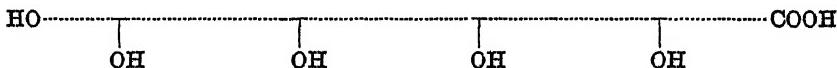
The thio aldehydes and ketones show more tendency to polymerize than their oxygen analogs (158). Organic silicon compounds of the types $R_2Si=O$, $RSi(=O)OH$, etc., are frequently incapable of being isolated in the monomeric condition, and they invariably polymerize very readily. It is interesting in this connection to compare carbon dioxide and silicon dioxide. The latter is probably a three-dimensional polymer.

V. POLYFUNCTIONAL REACTIONS AND NON-LINEAR POLYMERS

The polymers discussed thus far, whether formed by condensation or by self-addition, are of a type that may be symbolized by the formula -A-A-A-A-A-A-A-. . . . Reactions of condensation are not limited to bifunctional compounds, however. If one of two reactants contains two functional groups and the other contains more than two, the product will be not a simple chain but a more complicated structure. Such reactions may be called bi-trifunctional, bi-tetrafunctional, tri-trifunctional, etc. (18). Reactions of this class are especially important technically in connection with the formation of synthetic resins. Two examples may be considered.

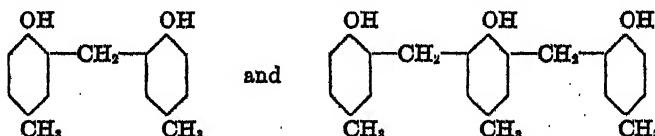
The glyptal resins are formed by the action of phthalic acid on glycerol. The reaction first leads to the formation of a fairly soft, soluble, thermoplastic resin, and this on being heated further yields a hard, insoluble resin which is completely lacking in thermoplasticity. The only reaction involved in the process is esterification (112), and the resin can be saponified completely to yield phthalic acid and glycerol (51). Analysis of the resin just before the infusible stage is reached shows that the glycerol and the phthalic acid are far from having reacted completely with each other, that is, free carboxyl and presumably free hydroxyl groups are still present. The behavior of a dihydric alcohol such as ethylene glycol in this reaction presents quite a different picture from glycerol. With the glycol it is possible to obtain complete esterification, but however far the reaction is carried the product does not become infusible or insoluble. The reason for

this difference is obvious. The polyester formed from the dibasic acid and the glycol is linear; as the reaction progresses the molecules grow, but the growth takes place only in one dimension. Similar chains formed in the reaction of glycerol with phthalic acid would bear hydroxyl groups



By reaction of these groups with phthalic acid the chains would be linked together, and thus a very complicated three-dimensional molecule would be built up. After a certain degree of complexity is reached, the possibility of molecular mobility no longer exists. It is conceivable that this cross-linking of the chains finally results in a mass that is essentially a single molecule. In any event it is easy to see how the possibility of further reaction disappears long before all the carboxyl and hydroxyl groups have a chance to participate. It is easy to see also why the action of any dibasic acid on glycerol always yields an amorphous resin, whereas the polyesters from dibasic acids and dihydric alcohols are frequently crystalline.

The formation of Bakelite from phenol and formaldehyde may also be classified as a tri-bifunctional reaction. The formaldehyde behaves as though it were $\text{HO}-\text{CH}_2-\text{OH}$, and it reacts no doubt largely at the *o*- and *p*-hydrogens of the benzene nucleus. With phenol itself the number of possible products even of quite low molecular weight is so great that no intermediate polymeric individuals can be isolated. On the other hand *p*-cresol has only two readily reactive positions. Thus it is possible as Koebner has shown (113) to isolate the compounds indicated below



as crystalline individuals, by causing formaldehyde and *p*-cresol to react in the appropriate ratios, and these can be used to build up still longer chains of the same series. The progressive hydrol-

ysis of silicic esters to silicon dioxide, already discussed in a previous paragraph, furnishes another example of the formation of a three-dimensional polymer.

Addition polymerization may also lead to the formation of three-dimensional structures. Thus acetylene reacts with itself under the influence of certain catalysts (not necessarily copper) with the formation of cuprene, an infusible and insoluble powder, and Staudinger suggests (2) that in this reaction the first step is the formation of unsaturated chains —CH=CH—CH=CH—CH=CH=CH— which subsequently combine with one another. The vulcanization of rubber probably also involves the cross-linking of the long chains through the agency of the unsaturated linkages present (39).

VI. NATURAL POLYMERS

1. *The association theory versus the structural theory*

The peculiar and difficult physical and chemical behavior of polymers has occasionally led to the suggestion that forces of a peculiar kind are involved in their formation. Thus Thiele in 1899 (132) suggested that perhaps in such materials as polystyrene the molecules of monomer are bound together merely by partial valences. Röhm in 1901 (115) concluded that the transformation of monomeric acrylic esters into the highly polymeric form is not chemical reaction but a kind of allotropic change. Later Schroeter (116, 117) suggested that the formation of dimeric ketene and of tetrassalicylide is due to the manifestation of an excess of peripheral external force about the monomeric molecules, and that the actual chemical structures of the monomeric molecules are not changed in the process. These particular suggestions are not tenable in the light of chemical evidence now available, but the association theory of polymeric structure reappeared about 1924 and was widely accepted as an explanation of the peculiarities of natural high polymers (118). According to one form of this theory, cellulose, for example, might be an anhydroglucose having the molecular formula $C_6H_{10}O_6$. This molecule, because of the unusual strain of its cyclic structure or for some other reason, is supposed to exhibit enormously exaggerated forces of association

or residual valence, and hence to behave physically as though it were a material of very high molecular weight. In the same way proteins might be built up by the mutual association of various small units, e.g., diketopiperazines.

In support of this theory various investigators showed that it was possible by freezing and boiling point methods to obtain small and rapidly shifting values for the molecular weights of polysaccharides, proteins, and rubber. Repetition of these determinations by other investigators proved, however, that the low results were due in most cases to errors in technic. Other support came from x-ray studies, which indicated that the unit cell of the crystal lattice of some high polymers is too small to contain a very large molecule. It was assumed at the time that a unit cell could not contain less than one molecule, but studies of known substances of high molecular weight proved that this assumption was incorrect. Meanwhile Staudinger's studies of synthetic materials repeatedly demonstrated that polymerization may lead to the formation of very long chains built up by real chemical forces in a regular fashion, and that such synthetic materials often resemble natural high polymers in many significant physical and chemical properties. Studies made in this laboratory on high polymers formed by condensation reactions led to the same conclusion. The idea that natural high polymers involve some principles of molecular structure peculiar to themselves and not capable of being simulated by synthetic materials is too strongly suggestive of the vital hypothesis, which preceded the dawn of organic chemistry, to be seriously considered.

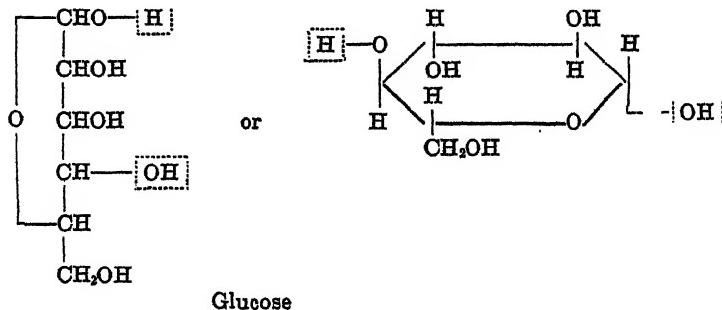
It should be emphasized in this connection that polymerization is not peculiar to unsaturated compounds, and that a very high degree of mobility in the relation between a monomer and its polymer does not preclude the intervention of real primary valence forces in the process or the presence of a definite macromolecular structure in the polymer. This fact is illustrated especially by adipic anhydride and the six-membered cyclic esters discussed in previous sections of this paper. These materials appear to exhibit all the supposedly diagnostic features of association polymerization; nevertheless their transformation into polymers

is a real chemical process, and the polymers are actually made up of large molecules. No example is yet known in which a small molecule of known structure simulates a material of high molecular weight without undergoing any change in structure.

A return to the simple structural theory of organic chemistry and the application of modern tools have been responsible, during the past five years, for very rapid progress in the interpretation of the structure and properties of some of the simpler, naturally occurring high polymers. It appears that many naturally occurring macromolecular materials have a linear polymeric structure. The present status of this subject has been reviewed by Meyer and Mark (3), and in the following paragraphs only cellulose and rubber are briefly discussed.

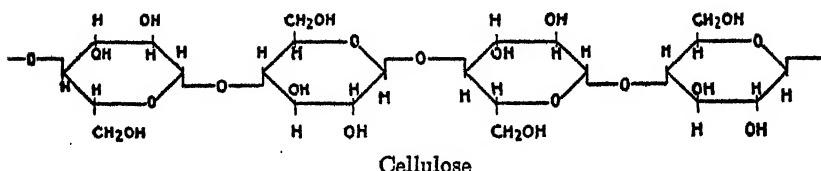
2. Cellulose

Chemical evidence for a linear polymeric structure in the cellulose molecule has long been available (125). More recently, Haworth's proof of the structure of cellobiose (120) and certain studies of the relation of glucose and cellobiose to cellulose (122) furnished the basis for tentative efforts to determine the nature of the units in the molecular chain (121). Sponsler and Dore (123) on the basis of x-ray evidence first put the linear polymeric structure in explicit form. This formula was further developed by Meyer and Mark (119), and it can now be said that the structure of cellulose, at least in its essential outlines, is definitely known. In its simplest chemical form the structural unit of cellulose may be represented by the bivalent radical derived by the removal of water from glucose (glucopyranose)



Glucose

A large number of these units are united chemically to form a long chain which constitutes the cellulose molecule.



(For a more detailed picture of the spatial arrangements in this chain, including the atomic distances, see reference 3.) In the cellulose fiber (cotton, ramie, etc.) these long molecules are lined up parallel with each other along the fiber axis.

To complete this formula it is necessary to specify the nature of the terminal groups and the length of the chain. The most reasonable assumption is that the chains are open and terminated by hydroxyl groups (alcoholic at one end and semi-acetal at the other), but no very definite experimental evidence on this point is available. Meyer and Mark estimate that the molecular chains are made up of from sixty to one hundred glucose units (molecular weight, 10,000 to 16,000), and they assume that the molecules are segregated into compact bundles (crystallites or micelles) from which the gross structure of the cellulose fiber is built up. Staudinger, however, has presented evidence (124) that the crystallites do not persist in dispersions of cellulose and its derivatives, and that the molecules have a weight much higher than 16,000. Stamm (126) has made direct measurements of particle size in ammoniacal copper dispersions of cellulose by the Svedberg ultracentrifugal method and obtained a value (on the copper-free basis) of $40,000 \pm 5$ per cent. The molecules in a given sample of cellulose are probably not all of the same length, but Stamm's data indicate a much higher degree of homogeneity than one might expect.

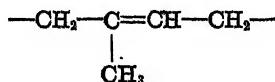
According to this picture cellulose, like other high polymers, is not a chemical individual in the sense of being composed of identical molecules, and its structure cannot be completely specified by a single exactly defined formula. On the other hand, this picture

accounts for the physical and chemical behavior of cellulose just as completely and satisfactorily as the formulas of simple compounds account for their properties. Cellulose fibers are very strong because of the parallel arrangement of the long molecular chains along the fiber axis. These chains adhere firmly to one another because of the cumulative force of association of the numerous hydroxyl groups. Strong aqueous alkali is able to penetrate this structure, and the resulting spreading apart of the chains causes lateral swelling. Other reagents (e.g., nitric acid) can penetrate the structure and esterify the hydroxyls without completely changing the apparent physical structure of the fiber. Cellulose is degraded much more rapidly by acids than by alkalies because the units are joined together by acetal linkages. The great length of the molecules accounts for the high viscosity of dispersions of cellulose and its derivatives. The very first stages of degradation greatly reduce the viscosity (124) because, for example, the hydrolytic absorption of 1 part of water in 2000 is capable of reducing by half the average size of the molecules. Undegraded cellulose has little or no reducing power (130) because it contains only one reducing group in an exceedingly large molecule, but reducing power is manifested and increases progressively with hydrolysis (124, 127). Complete hydrolysis finally gives a quantitative yield of glucose (125); cellobiose, a triose, a tetrose (128) and higher polysaccharides are formed as intermediate products. The hydrolysis agrees in its kinetics with the theoretical requirements for the chain structure (129). This structure also accounts for the presence of three esterifiable hydroxyl groups for each C₆ unit, and for the fact that completely methylated cellulose yields 2,3,6-trimethylglucose on hydrolysis (121).

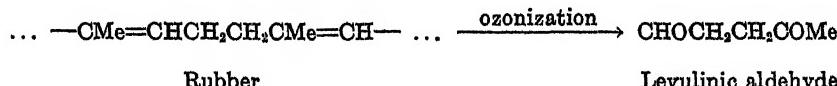
3. Rubber

It is well known that rubber hydrocarbon has the same empirical composition as isoprene (C_5H_8); that isoprene can be polymerized to yield a material resembling natural rubber; and that the behavior of rubber toward halogens, hydrobromic acid, and

hydrogen indicates the presence of one double bond for each five atoms of carbon. Harries many years ago (131) showed that the degradation of rubber by ozone yields chiefly levulinic acid and aldehyde, and this fact indicates that the rubber molecules must be largely built up by the repetition of the unit



in a regular manner as indicated below.



Harries first assumed that the rubber molecule is an eight-membered ring. Later the discovery of larger fragments in the products of degradation by ozone led him to suggest a larger cyclic structure. The physical properties of rubber clearly indicate however that it is macromolecular. Pickles (133) suggested the more plausible linear polymeric structure indicated above, and Staudinger (134) has brought forward a large mass of evidence in favor of this structure. The best evidence available indicates that the average weight of the rubber molecule is exceedingly large—perhaps in the neighborhood of 70,000 (135). It appears, moreover, that molecules having widely different sizes must be present in a given sample of rubber. Raw rubber is not homogeneous in its behavior toward solvents. When placed in contact with ether it swells and part of it diffuses into solution fairly rapidly. The action of fresh ether on the residue is much slower, and it is possible to carry the process of extraction so far as to obtain ultimately a residue that shows scarcely any tendency to dissolve. On the basis of such experiments it has been assumed that rubber is made up of two phases, sol and gel, and that the properties of rubber are due to the colloidal relationships of

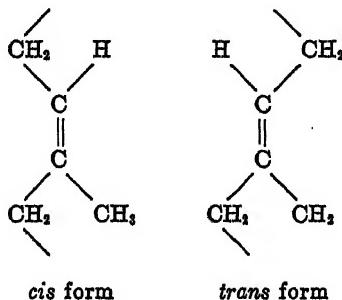
these two phases. In support of this idea there is the fact that colloidal dispersions of the jelly type frequently exhibit striking elasticity. This is true, for example, of polystyrene when it is swollen with unpolymerized styrene or with other hydrocarbon solvents. Whitby (137), however, has shown that sol-rubber (diffused) alone shows all the characteristic physical properties of raw rubber and differs from the latter only in degree. Moreover, it appears that sol- and gel-rubber are not distinct species and that neither of them is homogeneous. Rubber probably consists of a long continuous series of molecules of differing lengths. The smallest molecules dissolve quite readily and the largest ones only with difficulty.

If the rubber molecule is built up uniformly according to the regular plan indicated in formula II it should yield levulinic acid and aldehyde as the exclusive products of degradation by ozone. The yields of these products obtained by Harries accounted for only about 70 per cent of the rubber. Pummerer (136) has recently undertaken a reexamination of the Harries method, using carefully purified rubber, and has been able to account for 90 per cent of the rubber. He finds significant amounts of succinic acid, acetic acid, and acetone among the products. If the rubber molecule is an open chain, it might have an extra double bond at one end, and according to the disposition of this bond any of the above named by-products might be produced by ozonization. These products might also result from occasional irregularities in the arrangement of the units along the chain. The various possibilities have been outlined by Whitby (137).

Although the examination of unstretched rubber by x-rays gives only an amorphous ring, stretched rubber gives a sharp fiber diffraction pattern (138). When unstretched rubber is cooled in liquid air and then fractured by impact, it breaks up into irregular fragments; stretched rubber treated in the same way breaks up into thin fibers along the axis of stretch (139). These facts show that stretched rubber is much more highly oriented than unstretched rubber.

The structural unit of rubber contains a double bond and this

brings about the possibility of stereoisomerism. The units may be in the *cis* or the *trans* form (or both).



The x-ray data are said to favor the *cis* orientation (140). A spatial model of a long chain of such *cis* units is capable of being coiled up into a cylindrical spiral, and this spiral can be stretched out into a long chain. Various writers have suggested that the spiral model is capable of explaining the reversible stretching of rubber. This model has recently been discussed in detail by Fikentscher and Mark (140). It is assumed that the residual valence forces at the double bonds are responsible for holding the spiral in its compressed form. When the rubber is stretched, work is done against these forces, and the molecules assume the chain-like form, where they are much more highly oriented with respect to one another. Vulcanization is assumed to involve the chemical linking of these spiral chains at occasional points through sulfur atoms. A very small amount of combined sulfur does not interfere with the stretching of the spirals, but it prevents the chains from slipping past one another or being torn apart; consequently vulcanized rubber is not plastic, and it is not dissolved but only swelled by rubber solvents. As the amount of combined sulfur increases, the entire structure becomes more rigidly linked together, the plastic properties are completely suppressed, and the ability to imbibe solvent is lost. This picture is useful but it can hardly be said to account completely for the remarkable properties of rubber.

VII. THE PHYSICAL PROPERTIES OF HIGH POLYMERS

Perhaps the most important result of the study of synthetic high polymers has been to establish the fact that such materials are actually made up of exceedingly large molecules in the sense of the ordinary structural theory of organic chemistry. This is a point on which considerable scepticism has prevailed in the past, and the attempt to evade or ignore the idea of the molecules in dealing with high polymers has led to much speculative confusion.

It is true that synthetic linear high polymers are invariably mixtures whose molecules are chains of slightly differing lengths, and it is difficult to obtain reliable estimates of the average size of these molecules. Nevertheless it must be admitted that a molecule does not lose any of its definiteness as an entity either through the fact that it is exceedingly large or through the fact that it cannot be completely separated from other similar but slightly different molecules, and the properties of high polymers must ultimately be conditioned by the kinds of molecules which they contain.

It would be beyond the intended scope of this paper to attempt a detailed discussion of the relation between the molecular structure and the physical properties of high polymers, but there are two or three points that deserve some mention.

It is evident that in some respects the physical behavior of a molecule whose length is 100 times as great as its other dimensions must be profoundly different from that of a small compact molecule. Enormously long, flexible, and clumsy molecules must be very sluggish in their kinetic behavior, and it is not surprising that high polymers cannot be distilled or that they are never obtained in the form of thin mobile liquids.

The cohesive forces which resist the separation of molecules from one another (as measured, for example, by the heat of vaporization) increase continuously with increasing molecular weight in a given series, and in high polymers they reach values greatly in excess of the energy required to rupture a primary valence linkage in a chain (141). For this reason high polymers cannot be distilled without decomposition; indeed it appears that

the upper limit of distillability may lie at as low a molecular weight as 1200 to 1500 (44).

1. Solubility and colloidal properties

High polymers are subject to the same rough qualitative solubility rules that apply to simple compounds: like dissolves like; polar compounds dissolve in polar solvents and non-polar compounds in non-polar solvents; solubility in a given series diminishes with increasing molecular weight. Thus, rubber and polystyrenes are soluble in benzene, but not in acetone; polyamides are not dissolved by the usual organic solvents, but are dissolved by hot formamide; polyacrylic acid is soluble in water, while its esters dissolve in organic solvents but not in water; polystyrenes of low molecular weight (about 1000) are soluble in ether while polystyrenes of high molecular weight (about 20,000) are only slightly soluble in ether but are still dissolved by benzene.

The solubility of high polymers is sometimes surprisingly great compared with that of analogous simple compounds of much lower molecular weight. The higher normal paraffin hydrocarbons (e.g., heptacontane) are practically insoluble in any solvents at the ordinary temperature, while polystyrene and hydrorubber, which are essentially very long paraffin chains substituted at intervals by phenyl and methyl groups, dissolve readily in benzene. One reason for this no doubt lies in the fact that most high polymers are mixtures of molecules of different lengths, and these are capable to a certain extent of behaving independently in their solubility behavior. Moreover, the crystal lattices of high polymers are not so well ordered and rigidly constructed as those of low molecular weight materials and they may, for this reason, be more susceptible to attack by solvents.

The fact that the cohesive forces operating between large molecules are exceedingly high does not mean that polymers are incapable of forming molecular dispersions. Solubility depends upon specific affinities. Soluble linear polymers of relatively low molecular weight (e.g., 1000 to 5000) dissolve spontaneously and very rapidly in appropriate solvents and yield solutions which

are not highly viscous. The osmotic unit in these solutions is the molecule, not an aggregate of molecules. For various polyesters this has been proved (32, 34) by the fact that the same molecular weight values are obtained in a variety of solvents and by both freezing point and boiling point methods. Moreover, as the data of table 2 show, direct chemical determinations of molecular weight give the same values as the osmotic methods. Polyesters having molecular weights considerably above 5000 dissolve in the same solvents as the lower polyesters, but the process of solution is slower and the solutions are more viscous (51). The same behavior is observed in other polymeric series. Polystyrenes having molecular weights of about 1000 dissolve instantly in benzene, and the viscosity of the solutions is low; polystyrenes having molecular weights above 10,000 swell before dissolving, and the solutions are highly viscous (102). These evidences of colloidal behavior are due simply to the fact that the molecules are exceedingly long. The probable mechanism of solution of certain polymers is best illustrated by a specific example.

Rubber is made up of enormously long hydrocarbon chains ranging perhaps from 1000 to 10,000 Å. in length. These chains have a high specific affinity for certain non-polar solvents such as benzene. In a mass of rubber, adjacent chains are firmly bound to one another by cohesive forces. The structure is not an entirely regular one and there is no doubt a considerable amount of purely mechanical entanglement. Benzene, by virtue of its specific affinity for the chains, is capable of penetrating into the mass, solvating the chains, and spreading them apart. The structure thus becomes swollen and more tenuous, and finally individual fragments are carried away into solution. The fragments may be single molecules or only incompletely disrupted aggregates, but finally, if sufficient solvent is present, the latter are broken down and what amounts to an actual molecular dispersion results. This dispersion has a very high viscosity even when quite dilute, for the molecules are not only very large, but, owing to the fact that they are solvated and extended in only

one dimension, they have an effective radius of action quite out of proportion to their size.

The view that lyophilic dispersions of linear high polymers are usually true molecular dispersions, although it has not yet been generally accepted by colloid chemists (see 174), has been supported by Staudinger with a large mass of evidence (146, 102, 124, 135), which in its cumulative force seems to the writer fairly conclusive. Reference may be made also to Stamm's determination with Svedberg's ultracentrifuge of the particle size of cellulose dispersions in copper-ammonia solutions (126). He obtained the value $40,000 \pm 5$ per cent. It is quite certain on various grounds that the average molecular weight of cellulose cannot be less than about 16,000, so that if Stamm's particle is an aggregate it cannot contain more than two or three molecules. It seems highly arbitrary to assume that the solvent action of the dispersing agent, which depends upon a specific affinity for the cellulose molecules, should be capable of carrying off the molecules only as pairs or triplets and never as single molecules.

It is of course not contended that association never occurs in lyophilic solutions of high polymers, but merely that association occurs only as the result of some appropriate peculiarity in the molecular structure of the polymer, e.g., through the presence of recurring amide or carboxyl groups.

2. Crystallinity

Linear polymers, in spite of their lack of complete homogeneity and their high molecular weight, are by no means always amorphous. As indicated in table 1, all the polyesters derived from glycols of the series $\text{HO}(\text{CH}_2)_n\text{OH}$ and acids of the series carbonic, oxalic, succinic, etc., separate from solvents in the form of powders which show quite definite melting points. On the other hand, similar esters derived from phthalic acid are invariably transparent, amorphous resins. The ability to crystallize appears to require a high degree of linear symmetry in the structural unit. The presence of side chains such as methyl or phenyl groups on the units, and the random mixing of structural units, which occurs, for example, when polymers are prepared from a single glycol

and two different acids, diminish the tendency toward crystallinity. Thus, polyesters and polyamides derived from unsubstituted aliphatic compounds are crystalline, and so also are the polyoxymethylenes and polyethylene oxide, while polymers derived from vinyl compounds of the type $XCH=CH_2$, are usually amorphous. In these vinyl polymers the X group diminishes the linear symmetry of the chain; moreover in the formation of such polymers occasional inversions of the order of the units probably occur.

The behavior of ethylene succinate (molecular weight, 3000) on crystallization appears to be typical of many high molecular weight materials. It separates from a melt or from concentrated solutions in chloroform as doubly refracting microscopic spherulites which grow to what appear to be star-like clusters of needles. Further growth leads to frost-like patterns. The melt finally solidifies to an opaque porcelain-like mass. From dilute alcohol solutions ethylene succinate separates in very thin, discrete needles, but these lose their identity as soon as the solvent has evaporated. This behavior is highly characteristic of very long chains. It is reproduced in all its details by such diverse materials as triacetylinulin (150), *n*-heptacontane (51), and trimethylcellulose. The photomicrographs of crystalline trimethylcellulose presented by Hess (149) would serve equally well to represent ethylene succinate. Linear polymers in the form of microcrystalline powders have a pronounced tendency to become electrified, and they strongly adsorb considerable amounts of water vapor even when they show scarcely any tendency to dissolve in water.

The crystallization of linear polymers probably involves the parallel arrangement of the long chains into compact bundles, since this arrangement enables the molecules to exert their maximum cohesive force (151). Loose parallel swarms of molecules may also exist in melts or solutions of the polymers. Molecules of identical length might be arranged in bundles as shown in figure 1 (a), but especially with very long molecules, a less regular type of structure such as that shown in (b) might be produced. The arrangement shown in (b) has no sharp

boundaries and this defect would be exaggerated if the molecules were not all of the same length. Since the molecules of a specimen of high polymer are very long and have not all the same length the lattice bundles first formed must more nearly resemble (b) than (a). In the presence of solvent, such crystals might persist as discrete particles, but in the absence of a solvent they would tend to coalesce and lose their identity, owing to the absence of sharp boundaries and the incomplete neutralization of the residual forces of the projecting molecules. Thus, it is never possible to isolate large discrete (unsolvated) crystals of high polymers. Moreover, though solid masses of crystalline high polymers may be either hard and brittle or very tough, or soft and wax-like, they never show any definite planes of cleavage. The coalescence of the initial crystallites, which occurs as a molten mass of polyester finally solidifies, must occur in a random



FIG. 1a

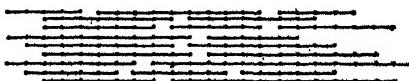


FIG. 1b

and rather disordered fashion, and it is probable also that the crystallites are cemented together by molecules that have not succeeded in completely identifying themselves with any particular crystallite.

The melting points of crystalline linear polymers show certain regularities. For a given molecular weight the melting points increase with the cohesive force (polarity) of the structural units. Polyamides have much higher melting points than analogous polyesters. Polyesters derived from short chain dibasic acids melt higher than those derived from the longer chain acids; mixed polyesters melt lower than simple ones. For a given polyester the melting point usually increases with increasing molecular weight up to a certain point, and after that it remains unchanged even though the molecular weight be increased many fold. Melting points are sometimes rather vague, but more frequently they are surprisingly sharp even when the molecular

weight is so high that the molten polymer shows no sign of flowing and the only indication of melting is the disappearance of opacity.

The question of the meaning of the term crystallinity in connection with high polymers is rather confused. Linear polyesters whose molecular weights lie below 5000 are definitely crystalline; they have sharp melting points and the crystals can actually be seen under the microscope. The evidences of crystallinity in polyesters whose molecular weights lie above 10,000 are somewhat more vague, but even these materials furnish sharp x-ray powder diffraction patterns. Similar though less sharply defined patterns are obtained from a transparent sheet of regenerated cellulose. These patterns indicate that part at least of the molecules of such materials must be definitely ordered with respect to one another. On the other hand, certain linear polymers, e.g., polystyrene, can be obtained in the form of white powders which show no microscopic or x-ray evidence of crystallinity. In these cases apparently the molecules tend to collect into discrete aggregates of some kind, but not in a sufficiently orderly fashion to exhibit any of the usual properties associated with crystals.

3. Mechanical properties

High polymers are very extensively used as structural materials in the construction of artifacts. One has only to mention cellulose, silk, and rubber to indicate the great economic importance of these non-chemical uses of organic materials. These uses depend upon such properties as mechanical strength, toughness, pliability, and elasticity. Such properties are found to a useful degree only among polymers of very high molecular weight. The synthetic materials of this class that have been most successfully used are three-dimensional polymers such as Bakelite and the glyptals. These materials have considerable strength, rigidity and toughness, but they are completely amorphous, and they are greatly inferior to natural fibers in breaking strength and pliability. The breaking strength of a flax fiber (100 kg. per sq. mm.) is of the same order as that of a good grade of steel (152). The qualities necessary for a useful fiber appear to be associated with a very high molecular weight linear polymeric structure and

a certain degree of crystallinity or definite order in the arrangement of the molecules. The relation between molecular structure and arrangement and the physical properties of fibers has been most clearly recognized and discussed by Meyer and Mark (3). In a natural cellulose or silk fiber the long molecular chains are arranged in an ordered fashion parallel with the fiber axis. This state of affairs is symbolized in figure 2. This arrangement provides the maximum possible strength in the direction of the fiber axis since the mutual cohesive force of the long chains is fully utilized. To rupture the fiber it is necessary to cause the chains to slip past one another against this cohesive force as indicated in the dotted line. A transparent sheet of regenerated cellulose shows (by x-ray patterns) a certain degree of order in the arrangement of its molecules, but there is no general orienta-



FIG. 2

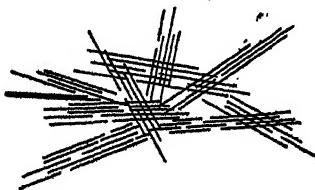


FIG. 3

tion. This state of affairs is symbolized in figure 3. This more or less random arrangement of ordered molecular aggregates can be brought into the more highly ordered state symbolized in figure 2 merely by the action of mechanical stress. Thus the strength of a sheet of Cellophane that initially has approximately the same strength in all directions can be so changed, merely by careful stretching, that its strength along the axis of stretch is increased several fold (153). At the same time its strength along the axis normal to its stretch is considerably diminished. The strength of a rayon filament can be increased several fold by the action of stress while it is in the spinning bath, and a comparison of the x-ray patterns shows a much higher degree of orientation along the fiber axis in the filament formed under stress. For a rough mechanical analogy of the mechanism of this process one may picture a disordered mass of long straws (molecules) coated

with a semi-fluid adhesive (cohesive force). The gradual application of stress to such a mass would finally bring the straws into parallel alignment where they would more strongly cohere and resist the further action of stress.

The peculiarities of high polymers are nowhere more strikingly exemplified than in this curious ability to accept permanent orientations through the action of mechanical stress. The properties of simple organic compounds are, generally speaking, independent of their physical history; they are completely determined by the nature of the molecules. Very large molecules, however, are not capable of adjusting themselves instantly to any changes in physical environment, and the properties of a very high molecular weight material may vary over a wide range depending upon the physical treatment it has received.

REFERENCES

- (1) STAUDINGER, H.: *Helv. Chim. Acta* **12**, 1183 (1929).
- (2) STAUDINGER, H.: *Z. angew. Chem.* **42**, 37, 67 (1929).
- (3) MEYER, K., AND H. MARK: *Der Aufbau der hochpolymeren organischen Naturstoffe*. Akademische Verlagsgesellschaft, Leipzig (1930).
- (4) STAUDINGER, H.: *Ber.* **59**, 3019 (1926).
- (5) SCHEIBER, J., AND K. SANDIG: *Die künstlichen Harze*. Wissenschaftliche Verlagsgesellschaft, Stuttgart (1929).
- (6) MEERWEIN, H.: *Houben's Die Methoden der organischen Chemie*, 3rd edition, Volume II, p. 593. Georg Thieme, Leipzig (1925).
- (7) MEYER, K.: *Naturwissenschaften* **16**, 781 (1928); *Z. angew. Chem.* **41**, 985 (1928).
- (8a) HILL, H. S., AND H. HIBBERT: *J. Am. Chem. Soc.* **45**, 3124 (1923).
- (8b) FRANKE, A., AND E. GIGERL: *Monatsh.* **49**, 8 (1928).
- (9) HESS, K.: *Die Chemie der Cellulose*, p. 574. Akademische Verlagsgesellschaft, Leipzig (1928).
- (10) USHAKOV, S. N., AND A. V. KON: *C. A.* **24**, 3796 (1930).
- (11) INGOLD, C. K., AND E. H. INGOLD: *J. Chem. Soc.* **1928**, 2249.
- (12) BERZELIUS, J. J.: *Jahresbericht* **12**, 63 (1833).
- (13) Reference 3, p. 47.
- (14) STAUDINGER, H., AND O. SCHWEITZER: *Ber.* **62**, 2395 (1929).
- (15) ROTHNER, E.: *Monatsh.* **15**, 665 (1894).
- (16) FAWORSKI, A.: *Chem. Zentr.* **1907**, I, 16.
- (17) LOURENÇO: *Ann. chim. phys.* **67**, 293 (1863).
- (18) CAROTHERS, W. H.: *J. Am. Chem. Soc.* **51**, 2548 (1929).
- (19) DIETZEL, R., AND R. KRUG: *Ber.* **58**, 1307 (1925).
- (20) WILLSTÄTTER, R., AND C. CRAMER: *Ber.* **43**, 2976 (1910).
- (21) GOLDSCHMIDT, S., E. SCHULZ, AND H. BERNARD: *Ann.* **478**, 1 (1930).
- (22) BUSCH, M., AND W. SCHMIDT: *Ber.* **62**, 2612 (1929).

- (23) WAGNER-JAUREGG, T.: Ber. 63, 3213 (1930).
 (24) BLAISE, E., AND A. KOEHLER: Compt. rend. 148, 1773 (1909).
 (25) RUIZICKA, L., AND M. STOLL: Helv. Chim. Acta 11, 1159 (1928).
 (26) JOHANSSON, H.: Lunds Universitets Årsskrift, II (2) 12, 3 (1916).
 (27) JOHANSSON, H.: Ber. 48, 1262 (1915).
 (28) BLAISE, E. E., AND L. MARCILLY: Bull. soc. chim. [3] 31, 308 (1904).
 (29) CHUIT, P., AND J. HAUSSER: Helv. Chim. Acta 12, 463 (1929).
 (30) LYCAN, W. H., AND ROGER ADAMS: J. Am. Chem. Soc. 51, 625, 3450 (1929).
 (31) BOUGALT, J., AND L. BOURDIER: Compt. rend. 147, 1311 (1908).
 (32) CAROTHERS, W. H., AND J. A. ARVIN: J. Am. Chem. Soc. 51, 2560 (1929).
 (33) CAROTHERS, W. H., AND F. J. VAN NATTA: J. Am. Chem. Soc. 52, 314 (1930).
 (34) CAROTHERS, W. H., AND G. L. DOROUGH: J. Am. Chem. Soc. 52, 711 (1930).
 (35) CAROTHERS, W. H., J. A. ARVIN, AND G. L. DOROUGH: J. Am. Chem. Soc. 52, 3292 (1930).
 (36) KERSCHBAUM, M.: Ber. 60, 902 (1927).
 (37) MARVEL, C. S., AND E. R. BIRKHEIMER: J. Am. Chem. Soc. 51, 260 (1929).
 (38) STAUDINGER, H.: Ber. 53, 1073 (1920).
 (39) MEYER, K., AND H. MARK: Ber. 61, 1948 (1928).
 (40) DAVIDOFF: Ber. 19, 406 (1886).
 (41) VORLÄNDER, D.: Ann. 280, 167 (1894).
 (42) TILITSCHAEW, M.: J. Russ. Phys. Chem. Soc. 57, 143 (1925); Chem. Zentr. 1926, I, 2667.
 (43) KIENLE, R. H., AND G. HOVEY: J. Am. Chem. Soc. 52, 3636 (1930).
 (44) CAROTHERS, W. H., J. W. HILL, J. E. KIRBY, AND R. A. JACOBSON: J. Am. Chem. Soc. 52, 5279 (1930).
 (45) FRANKE, A., AND O. KLENBERGER: Monatsh. 33, 1189 (1912).
 (46a) SCHLUBACH, H. H., AND E. C. GOES: Ber. 55, 2889 (1922).
 (46b) ZIEGLER, K., AND H. COLONIUS: Ann. 135, 474 (1900).
 (47) SCHORIGEN, P.: Ber. 43, 1931 (1910).
 (48a) GABRIEL, S.: Ber. 22, 3338 (1889).
 (48b) SCHOTTEN, C.: Ber. 21, 2240 (1888).
 (49a) CAROTHERS, W. H., AND G. J. BERCHET: J. Am. Chem. Soc. 52, 5289 (1930).
 (49b) GABRIEL, S., AND T. A. MAASS: Ber. 32, 1266 (1899).
 (50a) v. BRAUN, J.: Ber. 40, 1885 (1907).
 (50b) MANASSE, A.: Ber. 35, 1367 (1902).
 (50c) DIEHL, L., AND A. EINHORN: Ber. 20, 377 (1887).
 (51) Unpublished results.
 (52a) v. BRAUN, J., AND A. STEINDORFF: Ber. 38, 3083 (1905).
 (52b) v. BRAUN, J.: Ber. 43, 2853 (1910).
 (52c) v. BRAUN, J., AND C. MÜLLER: Ber. 39, 4110 (1906).
 (53a) LITTMANN, E. R., AND C. S. MARVEL: J. Am. Chem. Soc. 52, 288 (1930).
 (53b) KNORR, L., AND P. ROTH: Ber. 39, 1420 (1906).
 (54) STAUDINGER, H., AND E. OTT: Ber. 41, 2214 (1908).
 (55) VOERMAN, G. L.: Rec. trav. chim. 23, 265 (1904).
 (56) HILL, J. W.: J. Am. Chem. Soc. 52, 4110 (1930).
 (57) GRIGNARD, V., AND G. VIGNON: Compt. rend. 144, 1358 (1907).
 (58) HÜCKEL, W.: Fortschritte Chem., Physik. physik. Chem. 19, No. 4, 7 (1927).

- (59) LONSDALE, K.: Proc. Roy. Soc. (London) **123**, 494 (1929).
 (60) KING, G.: J. Oil Colour Chem. Assocn. **13**, 28 (1930).
 (61) LE FEVRE, J. W.: J. Chem. Soc. 1929, 733.
 (62) FERBER, E.: Ber. **62**, 183 (1929).
 (63) SMYTH, C. P., AND W. S. WALLS: J. Am. Chem. Soc. **53**, 527 (1931).
 (64) TILITSCHKEVICH, M.: J. Russ. Phys. Chem. Soc. **58**, 447 (1926); C. A. **21**, 3358 (1927).
 (65a) GANE, R., AND C. K. INGOLD: J. Chem. Soc. 1928, 1594.
 (65b) GANE, R., AND C. K. INGOLD: J. Chem. Soc. 1928, 2267.
 (66) MOHR, E.: J. prakt. Chem. **98**, 315 (1918).
 (67) STOLL, M., AND G. STOLL-COMTE: Helv. Chim. Acta **13**, 1185 (1930).
 (68) v. BRAUN, J., AND B. BAETSCH: Ber. **45**, 3376 (1912).
 (69) STANLEY, W. M., AND R. ADAMS: J. Am. Chem. Soc. **52**, 1200 (1930).
 (70) RUGGII, P.: Ann. **392**, 92 (1912).
 (71a) RUZICKA, L., M. STOLL, H. W. HUYSER, AND H. A. BOEKENOOGHEN: Helv. Chim. Acta **13**, 1153 (1930).
 (71b) RUZICKA AND COWORKERS: Helv. Chim. Acta **9**, 230, 249, 339, 389, 399, 499, 715, 1008 (1926); **10**, 695 (1927); **11**, 496, 670, 686, 1174 (1928).
 (72) FICHTER, F., AND A. BEISSWENGER: Ber. **36**, 1200 (1903).
 (73) HOLLO, E.: Ber. **61**, 895 (1928).
 (74) DREW, H. D. K., AND W. N. HAWORTH: J. Chem. Soc. 1927, 775.
 (75) BISCHOFF, C. A., AND P. WALDEN: Ann. **279**, 45 (1898).
 (76) BISCHOFF, C. A., AND P. WALDEN: Ber. **26**, 262 (1898).
 (77) BISCHOFF, C. A., AND P. WALDEN: Ann. **279**, 71 (1898).
 (78) CARTER, S. R., W. N. HAWORTH, AND R. A. ROBINSON: J. Chem. Soc. 1930, 2125.
 (79) JOHANNSSON, H., AND H. SIBELIUS: Ber. **52**, 745 (1919).
 (80a) BALBIANO, L.: Ber. **34**, 1501 (1901).
 (80b) BALBIANO, L., AND D. TRASCIATTI: Ber. **33**, 2323 (1900).
 (81a) MAILLARD, L. C.: Ann. chim. **1**, 519 (1914).
 (81b) MAILLARD, L. C.: Ann. chim. **2**, 210 (1914).
 (82) CURTIUS, T., AND A. BENBATH: Ber. **37**, 1279 (1904).
 (83) SHIBATA, K.: Acta. Phytochim. **2**, 39 (1925); **2**, 194 (1926).
 (84a) CURTIUS, T.: Ber. **37**, 1284 (1904).
 (84b) CURTIUS, T.: Ber. **16**, 753 (1883).
 (85) FISCHER, E.: Ber. **39**, 2924 (1906).
 (86) DELSPINE, M.: Bull. soc. chim. [4] **27**, 740 (1920).
 (87) GRICHKEVITCH-TROKHIMOVSKY, E.: Bull. soc. chim. [4] **24**, 541, 543, 546, 548, 549, 551 (1918).
 (88) KATZ, J. R.: Z. angew. Chem. **41**, 329 (1928).
 (89a) LEUCHS, H., AND W. GEIGER: Ber. **41**, 1721 (1908).
 (89b) LEUCHS, H.: Ber. **39**, 857 (1906).
 (90) CURTIUS, T., AND W. SIEBER: Ber. **55**, 1543 (1922).
 (91) COHEN, J. B.: Organic Chemistry, Part I, p. 193. Edward Arnold and Company, London (1928).
 (92) STANLEY, H. M.: J. Soc. Chem. Ind. **49**, 349T (1930).
 (93) MIGNONAC, G., AND R. V. DE SAINT-AUNAY: Bull. soc. chim. **47**, 522 (1930).
 (94) PLEASE, R. H.: J. Am. Chem. Soc. **52**, 1158 (1930).

- (95) LEBEDEV, S. V., AND G. G. KOSLYANSKII: Ber. 63, 1432 (1930).
- (96a) STAUDINGER, H., M. BRUNNER, K. FREY, P. GARBSCH, R. SIGNER, AND S. WEHRLI: Ber. 62, 241 (1929).
- (96b) STAUDINGER, H., K. FREY, P. GARBSCH, AND S. WEHRLI: Ber. 62, 2912 (1929).
- (96c) STAUDINGER, H., AND M. BRUNNER: Helv. Chim. Acta 12, 972 (1929).
- (96d) MILAS, N. A.: Proc. Natl. Acad. Sci. 14, 844 (1928).
- (97a) STAUDINGER, H., M. BRUNNER, AND W. FEIST: Helv. Chim. Acta 13, 805 (1930).
- (97b) FLUMIANI, G.: Z. Elektrochem. 32, 221 (1926).
- (98a) STAUDINGER, H., K. FREY, AND W. STARCK: Ber. 60, 1782 (1927).
- (98b) WHITBY, G. S., J. C. McNALLY, AND W. GALLAY: Trans. Roy Soc. Can. 22, 27 (1928).
- (98c) HERMANN, W. O., AND W. HAENNEL: Ber. 60, 1658 (1927).
- (99) STAUDINGER, H., AND E. URECH: Helv. Chim. Acta 12, 1107 (1929).
- (100) MOUREAU, C., AND C. DUFRAISSE: Ann. chim. 15, 158 (1921).
- (101a) STAUDINGER, H., A. A. ASHDOWN, M. BRUNNER, H. A. BRUSON, AND S. WEHRLI: Helv. Chim. Acta 12, 934 (1929).
- (101b) STAUDINGER, H., H. JOHNER, AND V. WIEDERSHEIM: Helv. Chim. Acta 12, 958 (1929).
- (101c) STAUDINGER, H., H. JOHNER, G. SCHIEMANN, AND V. WIEDERSHEIM: Helv. Chim. Acta 12, 962 (1929).
- (102a) STAUDINGER, H., AND W. HEUER: Ber. 63, 222 (1930).
- (102b) STAUDINGER, H., AND K. FREY: Ber. 62, 2909 (1929).
- (102c) STAUDINGER, H., AND R. MACHEMER: Ber. 62, 2921 (1929).
- (102d) STAUDINGER, H.: Kolloid-Z. 51, 71 (1930).
- (103a) STAUDINGER, H., E. GEIGER, AND E. HUBER: Ber. 62, 263 (1929).
- (103b) STAUDINGER, H., AND V. WIEDERSHEIM: Ber. 62, 2406 (1929).
- (104) STAUDINGER, H., AND F. BREUSCH: Ber. 62, 442 (1929).
- (105) WHITBY, G. S., AND M. KATZ: J. Am. Chem. Soc. 50, 1160 (1928).
- (106) STARKWEATHER, H. W., AND G. B. TAYLOR: J. Am. Chem. Soc. 52, 4708 (1930).
- (107) TAYLOR, H. S.: Private communication.
- (108a) SCHOTZ, S. P.: Synthetic Rubber. E. Benn, London (1926).
- (108b) PANKOW, M.: Kunststoffe 20, 219, 248 (1930).
- (109) MIDGLEY, T., JR., AND A. L. HENNE: J. Am. Chem. Soc. 52, 2077 (1930).
- (110a) STAUDINGER, H., R. SIGNER, H. JOHNER, M. LÜTHY, W. KERN, D. RUS-SIDIS, AND O. SCHWEITZER: Ann. 474, 145 (1929).
- (110b) STAUDINGER, H., AND M. LÜTHY: Helv. Chim. Acta 8, 41, 65, 67 (1925).
- (110c) STAUDINGER, H., H. JOHNER, R. SIGNER, G. MIE, AND G. HENGSTENBERG: Z. physik. Chem. 126, 425 (1927).
- (110d) STAUDINGER, H., AND R. SIGNER: Helv. Chim. Acta 11, 1047 (1928).
- (110e) MIE, G., AND J. HENGSTENBERG: Helv. Chim. Acta 11, 1052 (1928).
- (110f) STAUDINGER, H., AND R. SIGNER: Z. Krist. 70, 193 (1929).
- (110g) KOHLSCHÜTTER, H. W.: Ann. 482, 75 (1930).
- (110h) OTT, E.: Z. physik. Chem., Abt. B, 9, 378 (1930).
- (111a) CONANT, J. B., AND C. C. TONGBERG: J. Am. Chem. Soc. 52, 1659 (1930).

- (111b) BRIDGMAN, P. W., AND J. B. CONANT: Proc. Natl. Acad. Sci. 15, 680 (1929).
 (112) KIENLE, R. H., AND A. G. HOVEY: J. Am. Chem. Soc. 51, 509 (1929).
 (113a) KOESENER, M.: Chem.-Ztg. 54, 619 (1930).
 (113b) POLLAK, F., AND F. RIESENFIELD: Z. angew. Chem. 43, 1129 (1930).
 (114) TSCHUGAEFF, L.: Ber. 39, 3199 (1906).
 (115) RÖHM: Dissertation, Tübingen, 1901. Cited in reference 99.
 (116) SCHROETER, G.: Ber. 49, 2697 (1916).
 (117) SCHROETER, G.: Ber. 52, 2224 (1919).
 (118a) ABBERHALDEN, E.: Naturwissenschaften 12, 716 (1924).
 (118b) BERGMANN, M.: Naturwissenschaften 13, 1045 (1925).
 (118c) PRINGSHEIM, H.: Naturwissenschaften 13, 1084 (1925).
 (118d) BERGMANN, M.: Ber. 59, 2973 (1926).
 (118e) HESS, K.: Ann. 435, 1 (1924).
 (119a) MEYER, K. H., AND H. MARK: Ber. 61, 593 (1928).
 (119b) MARK, H., AND K. H. MEYER: Z. physik. Chem., Abt. B, 2, 115 (1929).
 (119c) MARK, H., AND G. V. SUSICH: Z. physik. Chem., Abt. B, 4, 431 (1929).
 (120) HAWORTH, W. N., C. W. LONG, AND J. H. G. PLANT: J. Chem. Soc. 1927, 2809.
 (121a) FREUDENBERG, K., AND E. BRAUN: Ann. 460, 288 (1928).
 (121b) FREUDENBERG, K.: Ann. 461, 130 (1928).
 (122a) IRVINE, J. C.: Chem. Reviews 1, 41 (1924).
 (122b) FREUDENBERG, K.: Ber. 54, 767 (1921).
 (123a) SPONSLER, O. L., AND W. H. DORE: Colloid Symposium Monograph 4, 174 (1926). Chemical Catalog Company, New York.
 (123b) SPONSLER, O. L.: Nature 125, 633 (1930).
 (124a) STAUDINGER, H., AND O. SCHWEITZER: Ber. 63, 3122 (1930).
 (124b) STAUDINGER, H., AND O. SCHWEITZER: Ber. 63, 2317 (1930).
 (124c) STAUDINGER, H., AND H. FREUDENBERGER: Ber. 63, 2331 (1930).
 (125) WILLSTÄTTER, R., AND L. ZECHMEISTER: Ber. 46, 2401 (1913).
 (126) STAMM, A. J.: J. Am. Chem. Soc. 52, 3047 (1930).
 (127) BERGMANN, M., AND H. MACHEMER: Ber. 63, 316 (1930).
 (128) WILLSTÄTTER, R., AND L. ZECHMEISTER: Ber. 62, 722 (1929).
 (129a) KUHN, W.: Ber. 63, 1503 (1930).
 (129b) FREUDENBERG, K., W. KUHN, W. DÜRR, F. BOLZ, AND G. STEINBRUNN: Ber. 63, 1528 (1930).
 (130) BERGMANN, M., AND H. MACHEMER: Ber. 63, 2304 (1930).
 (131) HARRIES, C.: Ber. 37, 2708 (1904); 38, 3985 (1905).
 (132) THIELE, J.: Ann. 306, 92 (1899).
 (133) PICKLES, S. S.: J. Chem. Soc. 98, 1085 (1910).
 (134a) STAUDINGER, H., AND J. FRITSCHI: Helv. Chim. Acta 5, 785 (1922).
 (134b) STAUDINGER, H.: Ber. 57, 1203 (1924).
 (134c) STAUDINGER, H., AND W. WIDMER: Helv. Chim. Acta 7, 842 (1924).
 (134d) STAUDINGER, H.: Z. angew. Chem. 38, 226 (1925).
 (134e) STAUDINGER, H., AND E. GEIGER: Helv. Chim. Acta 9, 549 (1926).
 (134f) STAUDINGER, H.: Kautschuk 1925, 5.
 (134g) STAUDINGER, H.: Kautschuk 1927, 63.
 (134h) STAUDINGER, H., M. ASANO, H. F. BONDY, AND R. SIGNER: Ber. 61, 2575 (1928).

- (134i) STAUDINGER, H., H. F. BONDY, AND E. GEIGER: Ann. 468, 1 (1929).
 (134j) STAUDINGER, H., AND H. F. BONDY: Ann. 468, 1 (1929).
 (134k) STAUDINGER, H.: Kautschuk 5, 94, 126 (1929).
 (134l) STAUDINGER, H., AND H. F. BONDY: Ber. 62, 2411 (1929).
 (134m) STAUDINGER, H., AND H. F. BONDY: Ber. 63, 724 (1930).
 (134n) STAUDINGER, H., AND E. O. LEUPOLD: Ber. 63, 730 (1930).
 (135a) STAUDINGER, H., AND H. F. BONDY: Ber. 63, 734 (1930).
 (135b) STAUDINGER, H.: Ber. 63, 921 (1930).
 (135c) STAUDINGER, H., AND H. JOSEPH: Ber. 63, 2888 (1930).
 (135d) STAUDINGER, H.: Kautschuk 6, 153 (1930).
 (135e) STAUDINGER, H., AND H. F. BONDY: Ber. 63, 2900 (1930).
 (136) PUMMERER, R.: Z. angew. Chem. 43, 757 (1930); PUMMERER, R., G. EBERMAYER, AND K. GERLACK: Ber. 64, 809 (1931).
 (137) WHITBY, G. S.: Trans. Inst. Rubber Ind. 5, 184 (1929); 6, 40 (1930).
 (138a) KATZ, J. R.: Chem.-Ztg. 49, 353 (1925).
 (138b) MEYER, K. H., AND H. MARK: Ber. 61, 1939 (1928).
 (139) HOCH, L.: Gummi-Ztg. 39, 1740 (1925).
 (140) FIXENTSCHER, H., AND H. MARK: Kautschuk 6, 2 (1930); Rubber Chemistry and Technology 3, 201 (1930).
 (141) DUNKEL, M.: Z. physik. Chem., Abt. A, 138, 42 (1928).
 (142) WHITBY, G. S., J. C. McNALLY, AND W. GALLAY: Trans. Roy. Soc. Can. 22, 27 (1928).
 (143) LEE, W. B., AND P. J. VAN RYSELBERGE: J. Phys. Chem. 33, 1543 (1929).
 (144) KATZ, J. R.: Z. angew. Chem. 42, 828 (1929).
 (145) RUZICKA, L., M. STOLL, H. W. HUYSER, AND H. A. BOEKENOOGHEN: Helv. Chem. Acta 13, 1152 (1930).
 (146a) STAUDINGER, H.: Kolloid-Z. 53, 19 (1930).
 (146b) SIGNER, R.: Z. physik. Chem., Abt. A, 150, 257 (1930).
 (147) MEYER, V., AND P. JACOBSON: Lehrbuch der organischen Chemie, zweiter Band, dritter Teil. Walther de Gruyter and Company, Berlin and Leipzig (1920).
 (148) WITTIG, G.: Stereochemie, p. 277. Akademische Verlagsgesellschaft, Leipzig (1930).
 (149) Reference 9, p. 432.
 (150) PRINGSHEIM, H., AND W. G. HENSEL: Ber. 63, 1096 (1930).
 (151) Reference 8, p. 50.
 (152) Reference 8, p. 153.
 (153) MARK, H.: Mellands' Textilber. 10, 695 (1929).
 (154a) ZAPPI, E. V.: Bull. soc. chim. 19, 249 (1916).
 (154b) v. BRAUN, J., AND W. SOBECKI: Ber. 44, 1918 (1911).
 (155) GRIGNARD, V., AND TCHEOUFAKI: Compt. rend. 188, 357 (1929).
 (156) KONRAD, E., O. BÄCHLE, AND R. SIGNER: Ann. 474, 276 (1929).
 (157) KIPPING, F. S., A. G. MURRAY, AND J. G. MALTBY: J. Chem. Soc. 1929, 1180.
 (158) SCHÖNBERG, A.: Ber. 62, 195 (1929).
 (159) Brit. patents, 279,406 and 314,524.
 (160) MORGAN, G. T., AND F. H. BURSTALL: J. Chem. Soc. 1930, 1497.
 (161) MANSFIELD, W.: Ber. 19, 696 (1886).

- (162) V. BRAUN, J., AND A. TRÜMPFLER: Ber. 43, 547 (1910).
- (163) V. BRAUN, J.: Ber. 43, 3220 (1910).
- (164) WALLACE, O.: Ann. 312, 205 (1900).
- (165) LIND, S. C., AND G. GLOCKLER: J. Am. Chem. Soc. 51, 2811 (1929).
- (166a) HARKINS, W. D., AND D. M. GANS: J. Am. Chem. Soc. 52, 5165 (1930).
- (166b) AUSTIN, J. B., AND I. A. BLACK: J. Am. Chem. Soc. 52, 4552 (1930).
- (167a) ZELINSKY, N.: Ber. 57, 264 (1924).
- (167b) FISCHER, F., F. BAUGERT, AND H. PICHLER: Brennstoff-Chem. 10, 279 (1929).
- (167c) PEASE, R. N.: J. Am. Chem. Soc. 51, 3470 (1929).
- (167d) SCHLÄPFER, P., AND M. BRUNNER: Helv. Chim. Acta 13, 1125 (1930).
- (168a) SABATIER, P., AND J. B. SENDERENS: Bull. soc. chim. 25, 683 (1901).
- (168b) Brit. patent, 303,797.
- (168c) SCHLÄPFER, P., AND O. STADLER: Helv. Chim. Acta 9, 185 (1926).
- (169a) LIND, S. C., AND R. S. LIVINGSTON: J. Am. Chem. Soc. 52, 4613 (1930).
- (169b) BATES, J. R., AND H. S. TAYLOR: J. Am. Chem. Soc. 49, 2438 (1927).
- (170a) McLENNON, J. C., M. A. PERRIN, AND J. C. IRETON: Proc. Roy. Soc. (London) 125A, 246 (1929).
- (170b) COOLIDGE, W. D.: Science 62, 441 (1925).
- (171) LIND, S. C., D. C. BARDWELL, AND J. H. PERRY: J. Am. Chem. Soc. 48, 1556 (1926).
- (172) MIGNONAC, G., AND R. V. DE SAINT-AUNAY: Compt. rend. 188, 959 (1929); Bull. soc. chim. 47, 14 (1930).
- (173) HUNTER, W. H., AND G. H. WOOLETT: J. Am. Chem. Soc. 43, 135 (1921).
- (174a) Reference 3, p. 173.
- (174b) KRAEMER, E. O., AND G. R. SEARS: J. Rheology, in press.
- (174c) LOEWEN, H.: Kautschuk 7, 12 (1931).
- (174d) MARK, H.: Kolloid-Z. 53, 32 (1930).

THE ENERGETICS OF HETEROTROPHIC BACTERIA¹

P. W. WILSON AND W. H. PETERSON

Departments of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin, Madison

Received May 29, 1930; revised to March 29, 1931

INTRODUCTION

D'Arcy Thompson has said, "When science wishes to speak plainly, she uses the language of mathematics." Without denying the truth of this statement, biologists in general have been skeptical of the practicability of the application of mathematics to the chemistry of the cell. The first attempts to apply the theory and practice of thermodynamics to cell phenomena met with considerable opposition; proponents of a vitalistic philosophy were loud in argument and long in speech with regard to the fallaciousness of the application of knowledge gained in experimental mechanics and thermochemistry to life processes.

The researches of Lavoisier and Laplace, Dulong and Depretz, Berthelot and Rubner definitely established that the first law of thermodynamics—the law of the conservation of energy—was fulfilled in the realm of biology as well as physics. The conception of the cell as a machine capable of transforming chemical energy into work and heat soon followed. But a necessary consequence of this conception postulated that the second law—the entropy of an isolated reacting system increases, the free energy decreases—also was satisfied by the organism. The latter was not so easy to demonstrate; many still thought that a special form of energy characteristic of protoplasm was concerned in the energy transformations of the cell. This "vital energy" did not necessarily obey the laws of classical thermodynamics. Apparent examples of departure from the second law exhibited by colloidal solutions strengthened this view, and it was even

¹ Herman Frasch Foundation Research in Agricultural Chemistry, Paper No. 7.

proposed that bacteria and other microorganisms might serve as "Maxwell demons" in the transfer of energy to a higher potential without work being supplied from a source outside the system. Because of these and other philosophical implications, the question of the validity of the second law in the realm of biology has engaged the attention of numerous writers. For reviews of this aspect of the subject the reader is referred to the papers of Meyerhof (48), Höber (29), Wachtel (69), Oppenheimer (53) and Guye (23).

The idea that the second law of thermodynamics was of a statistical nature and that entropy was a measure of the probability of a displaced equilibrium readjusting itself did much to elucidate the difficulties attendant on the anomalous behavior of microscopic bodies. The statistical nature of the law admits exceptions in a small population, but if the numbers approach those attained in gas volumes or in bacterial cultures, these deviations are eliminated as far as the gross effects are concerned. A discussion of the possible limitation of thermodynamical treatment of minute particles from the point of view of probabilities is given by Donnan (17) and Guye (23).

However certain we may be concerning the validity of the laws of thermodynamics in biological processes, the study of the energy relations of the cell offers experimental difficulties that to date have defied investigations other than those that deal with mass changes. As Linhart (41) has pointed out, the biologist in presenting his problems to the physical chemist is usually met with the statement that "biological processes are so highly complex that they are not susceptible to thermodynamic treatment." In a consideration of the cell as a machine operating in an isothermal manner to perform work, to liberate heat, and to synthesize substances of high energy content from materials of low energy value, it soon becomes obvious that complicated reactions occur and these reactions are coupled in a manner that is completely absent in the relatively simple, isolated systems usually studied in thermochemistry. However discouraging the outlook may be, some progress, partly of an experimental and partly of a theoretical nature, has been made.

The more general aspects of the energetics of the cell have been considered in the texts of Meyerhof (48), Oppenheimer (53), and Höber (29). Also Kruse (36) devotes part of a chapter of his "Allgemeine Mikrobiologie" to a treatment of the energetics of microorganisms, and the more recent texts of Buchanan and Fulmer (9) and Stephenson (63) discuss certain aspects of this field of bacteriology. Bacteria have been conveniently separated into two classes as regards the source of their energy and its utilization—the autotrophic and the heterotrophic. The former obtain their energy by oxidation of ammonia, nitrites, sulfur, etc., and perform true thermodynamical work in the synthesis of carbohydrate material from carbon dioxide. The heterotrophs, which comprise by far the majority of the known organisms, obtain their energy by oxidation or dissimilation of organic matter. Because of the relative simplicity of the processes involved in the metabolism of the autotrophs, much more attention has been given to the energetics of these organisms than has been devoted to the energy changes of the more complex decompositions brought about by the heterotrophic forms. The energetics of the first group have been adequately discussed by Meyerhof (43), Waksman (70), Buchanan and Fulmer (9), and Baas-Becking and Parks (2). This review will deal only with the second group, and to some extent with the yeasts, since the metabolism of the latter is very similar to that of many of the bacteria considered.

For convenience, this discussion is divided into three parts: (1) the general problem of energetics, which deals with the methods of study and with the source and possible utilizations of the energy available to the organism; (2) the application of energetics to typical fermentation processes; (3) the energetics of nitrogen fixation by bacteria.

I. GENERAL PROBLEM OF ENERGETICS IN BACTERIOLOGY

Methods used

Thermochemistry. In the field of bacterial energetics, the pioneer work of Rubner pointed the way in methods as well as in

the interpretation of results. In his first studies (57, 59), he made use of what might be termed "differential calorimetry" to gain an idea of the energy changes taking place in the growth of bacteria. The method, laborious and subject to numerous errors, was simply the determination of the calorific value of the medium before and after growth of bacteria. From the difference he calculated the energy necessary for the maintenance of the cell. The bacteria were removed by precipitation with iron, and the calorific value of the cells was estimated. From these data

TABLE I
Energy changes produced by growth of certain bacteria
Rubner

ORGANISM	INCREASE IN BACTERIAL NITROGEN milligrams	LOSS OF ENERGY BY MEDIUM kilogram- calories	ENERGY VALUE OF BACTERIAL CELLS kilogram- calories	PER CENT*	ENERGY OF MAINTEN- ANCE PER GRAM BACTERIAL NITROGEN PER DAY kilogram- calories
<i>B. pyocyanus</i>	190	24.70	6.84	27.7	15.6
<i>B. coli</i>	69	7.86	2.42	30.8	18.1
<i>B. proteus</i>	105	15.58	3.26	20.9	19.4
<i>B. thermophilus</i>	79	18.15	4.68	24.9	34.5
<i>B. typhosus</i>	42	11.18	1.30	11.6	42.8
<i>V. cholerae</i>	61	19.69	3.35	17.0	42.7
<i>B. diphtheriae</i>	35	13.48	1.63	12.1	60.6

* Percentage of energy lost by medium.

he could estimate the percentage of total energy that "disappeared" which reappeared in the cellular crop. Naturally this method was limited to a study of those organisms that did not produce gases (other than carbon dioxide) in their metabolism. Table 1 taken from his data gives typical results.

It will be noticed that those organisms which did not grow so well had high values for the energy of maintenance and low values for the percentage of the total energy changed that appeared in the organisms. The same medium (6 per cent beef extract) was used for all the organisms. It is probable that it was not suited to the physiology of some of the bacteria, and as a

consequence a higher expenditure of energy per unit weight resulted than is actually necessary for the functioning of that particular organism. A repetition of the experiment employing the medium best suited to the individual organism might show entirely different results.

It is of interest to compare the values obtained for bacteria with those of other species investigated by Rubner. The values are shown in table 2. Rubner attributed the lower values for the more complex organisms to the fact that by reason of their organization the liberated energy is used more economically.

TABLE 2
Energy of maintenance of various species
Rubner

SPECIES	ENERGY OF MAINTENANCE PER GRAM NITROGEN PER DAY kilogram-calories
Man.....	1.0
Dog.....	3.28
Mouse.....	15.19
Sparrow.....	17.4
Cold-blooded animals.....	0.4-0.6
Yeast.....	38.77
Bacteria.....	15.6-60.6

Using *B. proteus* as the experimental organism, Rubner (59) investigated the influence of temperature, age, and other variables on the energy changes. Temperature changes from 14°C. to 36°C. made little difference in the total energy change, but the rate was somewhat slower at the lower temperature. In the earlier stages of the fermentation (1 to 10 days), the total energy expended was two to three times larger than that during the later stages. Rubner attributed this to the difference of the energy requirement of the organism during the growth phase and during the "resting" period.

Tangl (65) also studied bacterial energetics as part of the problem of the energetics of ontogenesis, i.e., the energy changes coincident with the development of an individual. He used the

method of "differential" calorimetry and estimated the energy expended in the synthesis of 1 gram of dry bacterial substance. The results of one experiment gave the following values: *B. anthracis*, 8.6 kg-cal.; *B. suis*, 16.5 kg-cal.; *B. subtilis*, 12.5 kg-cal. The potential energy of the nutrients was 4.3 kg-cal. per gram and that of the cells 4.5 kg-cal.; yet in the various reactions that accompanied the synthesis of 1 gram of cell protoplasm, there disappeared from the medium nutrients equivalent to from 8.6 kg-cal. to 16.5 kg-cal. Tangl believed that little of this energy was actually used in cell synthesis.

Calorimetry. Due to the limitation of the differential calorimetric method, Rubner (59) developed a technique for following energy changes during growth of organisms by measuring the actual heat produced. He constructed a "bio-microcalorimeter" that would detect energy changes of the order of one to two gram-calories and followed the heat production of pure and mixed bacterial cultures on a variety of media. With *B. coli* under anaerobic conditions, he found that 68 per cent of the total energy expended appeared as heat and with *B. proteus* only 15 to 25 per cent. Unfortunately, the data for the total energy used were not from the same experiment in which the heat production was measured, hence these figures can be regarded only as approximate, and are very likely too low. Since Rubner's work, the technique of measuring heat production by means of the calorimeter has been greatly improved and a check on some of his findings would appear to be desirable. Hill (27) has developed a very sensitive differential microcalorimeter in which heat production is measured by a thermocouple instead of a Beckmann thermometer. By the use of this calorimeter, Shearer (62) showed that the heat production of *B. coli* was seven to eight times as great in a glucose-peptone broth as in a broth of the tryptic digest of casein, and that the heat produced by *B. coli* in casein digests was inversely proportional to the length of digestion. He attributed these results to a more economical utilization of energy by *B. coli* in the presence of amino acids. Unfortunately, no data are given for the heat production in glucose-tryptic digest of casein broth, hence the influence of the glucose on the economy was not

determined. Bayne-Jones (3, 4) has recently described a differential microcalorimeter of the Hill type especially adapted to bacterial fermentations and has studied heat production by *B. coli* and *Staph. aureus* during the various growth phases. The data presented indicated that during the logarithmic growth phase of *B. coli*, the heat production was a linear function of the time. As pointed out by Wetzel (72), this suggests that the rate of heat production per cell is not constant but diminishes exponentially with time. Further contributions concerned with heat production in other growth phases are necessary before a satisfactory interpretation of the significance of this observation can safely be made.

Meyerhof (44) studied heat production of bacteria in comparison with oxygen uptake. The heat produced was measured in a microcalorimeter in which 0.01° rise on the Beckmann thermometer corresponded to 2.5 g-cal. Parallel determinations of the oxygen uptake were made on the same culture in a Warburg microrespirometer or by Winkler's titration method. The quotient $\frac{\text{g-cal. produced}}{\text{mg. O}_2 \text{ consumed}}$ was used as a measure of the metabolism.

Meyerhof states this should be 3.2 for protein, 3.4 to 3.5 for carbohydrate, and 3.3 for fat. He grew *Vibrio Metchnikoff* in a medium that was not suited to the physiology of the organism, and found this quotient to be 3.95 with an error of 10 to 15 per cent. In a medium that allowed good growth, this quotient rose in one case to 4.1, and in another medium to 4.5. Meyerhof concluded that energy-liberating processes other than oxidations took place in this organism, since with blood cells, sea urchin eggs, etc., the quotient was constant irrespective of whether growth was retarded or not. Dissimilation processes, neutralization of acids and heat of solution of metabolic products might account for some of the excess energy.

Thermodynamics. Aside from the practical methods for the estimation of energy changes of the cell, a method is available that is more theoretical in character. This is an application of thermodynamics to the cell processes. Although there are obvious limitations to this method, and its free use of mathematics

discourages many biologists, it offers a tool for investigation that should not be disregarded. As long as the proper skepticism is maintained toward any data which cannot be directly verified in the laboratory, the danger that arises from the use of thermodynamics or any other theoretical aid is often far outweighed by the information given. For example, the judicious use of thermodynamics will often prove of value in the test of proposed mechanisms for unknown processes, and while thermodynamics is not concerned with any particular mechanism, its use may help to decide the most probable one. If a mechanism for a given biological process is known, the use of thermodynamics enables the experimenter to gain some idea of the energy available for work that arises from the process. For some time these advantages have been utilized by bacteriologists in a manner that is inaccurate and which takes no cognizance of the fact that the concentration of the components of a reaction affects the quantity of available energy liberated. It is a common practice to determine the metabolic products of an organism, to construct equations for the chemical changes involved, and, from thermochemical data (heats of combustion and formation), to determine the energy "available" to the organism. This procedure is often only a rough approximation, as the following discussion will show.

It is not in the scope of this paper to detail the chemistry and mathematics involved in the subject of thermodynamics as applied to chemical reactions. For a thorough presentation of the subject, Lewis and Randall's text (38) may be consulted, but for the biologist who wishes to avoid the more detailed application, the briefer treatment of the material by Buchanan and Fulmer (9) and by Fulmer and Leifson (19) is probably sufficient. However, two fundamental equations with their meaning should be considered here. In the calculation of energy changes prior to the Nernst (51), or the Lewis and Randall systems of thermodynamics, the criterion of the driving force of a reaction was the heat of reaction ($-\Delta H$). However, these authorities, following Gibbs, have shown that the change in free energy ($-\Delta F$) and not the heat of reaction ($-\Delta H$) is the true criterion. Free energy is the quantity of energy liberated in a reaction that is

available for doing work. $-\Delta F$ may be greater or less than $-\Delta H$ depending on the temperature coefficient of the former. If this temperature coefficient is negative, the free energy is less than the heat of reaction and *vice versa*. In the former case, the energy not available for work $T \left(\frac{\partial \Delta F}{\partial T} \right)_P$ is that part of the energy that must disappear as heat in order that the reaction may proceed. In case $-\Delta F$ is greater than $-\Delta H$, the excess energy is taken from the surroundings as heat and converted into a form capable of the performance of work. Hill (28) has emphasized the importance of the concept of free energy in biology and has discussed a few reactions which are endothermic if the heat of reaction is used as a criterion, but which can be used to do work on account of the conversion into free energy of heat from the environment. The relations between ΔF and ΔH are given by the following equations:

$$\Delta F = \Delta H - T \Delta S$$

or

$$\Delta F = \Delta H + T \left(\frac{\partial \Delta F}{\partial T} \right)_P$$

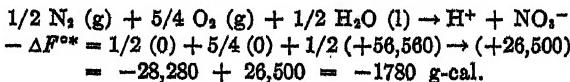
where ΔS is the change in entropy, T is the absolute temperature, and $\left(\frac{\partial \Delta F}{\partial T} \right)_P$ is the temperature coefficient of free energy at constant pressure. By reason of certain conventions of signs, a reaction is said to occur spontaneously, i.e., with liberation of free energy, when ΔF is negative. It must be recognized that $-\Delta F$ as defined above is the maximum energy available for work, and the actual work done will depend on the efficiency of the process involved. For a strictly reversible system, e.g., a galvanic cell nicely balanced, an efficiency of 100 per cent is possible, but in more practical applications such as the storage battery the efficiency is less and part of the free energy disappears as heat.

A second factor, which is of the utmost importance to the biologist, but which is often overlooked, is the change in $-\Delta F$ with changes in concentrations, or more accurately with activities

of the reactants and resultants. The value of $-\Delta F$ for a particular substance given in the literature is usually that for the element or compound when present in the standard state. The standard state is defined as the state in which the activity of the substance is unity. Thus at 25°C. and under 1 atm. pressure solid glucose, liquid water, gaseous oxygen and a molal solution of a solute (with proper correction for deviations from a perfect solution) are in the standard state. If a substance is transferred from its standard state to some other state, as by change of temperature, pressure, or concentration, energy changes are involved. It follows that if the reactants or resultants of a given reaction are present in a state other than the standard, the calculated free energy change must be corrected, since the calculation is based on the reaction in which each component appears in the standard state. The correction is given by the equation

$$\Delta F = \Delta F^\circ + RT \ln K,$$

where ΔF is the increase in free energy under the conditions studied, ΔF° equals the increase in standard free energy, R is the gas constant, T is the absolute temperature, and K is the equilibrium constant of the reaction. An example taken from Burk (11), dealing with fixation of nitrogen by legumes, may serve to make this clear.



* In this paper ΔF° refers in all cases to ΔF° at 25°C.

But if this reaction takes place at the concentrations that are likely to exist in the plant (legume), a different result is obtained, viz.: $\text{HNO}_3 = 0.0001 M$., $\text{O}_2 = 0.2 \text{ atm.}$, and $\text{N}_2 = 0.8 \text{ atm.}$

$$\therefore K = \frac{(0.0001)^2}{(0.8)^{1/2} (0.2)^{1/4}} = 8.6 \times 10^{-8}$$

$$\log K = -7.07$$

$$-\Delta F = -1780 + 1365 (-7.07) = 7870 \text{ g-cal.}$$

In this case it is observed that owing to change in concentrations (and hence activities) the sign of $-\Delta F$ is changed, and whereas the reaction would require energy from an outside source if each substance is present in its standard state, it will proceed spontaneously when the concentrations are changed.

In conclusion it must be noted that for precise work K must be calculated from the activities of the substances involved. However, concentrations may be used for this calculation in most of the ranges found in biological studies, where because of the complexity of the system, activities are often difficult to determine.

Baron and Polányi (5) were among the first workers to apply thermodynamics to biological processes. They made use of the Nernst heat theorem (51), which is not quite as accurate as the more recently developed system of Lewis and Randall. Considering animals in which the energy changes were due to the combustion of protein, fat, or carbohydrate, to the change of carbohydrate into fat, and to the production of work, Baron and Polányi calculated the free energy changes of these processes and made a balance which showed that the change in free energy could be positive only when heat is absorbed from the environment. In homiotherms the opposite takes place, consequently the total metabolism of these species results in a decrease in free energy. This means that at least in this case of biological energetics the second law of thermodynamics is valid. Experiments with poikotherms, e.g., bacteria, show that their total metabolism always results in a liberation of heat. Therefore it appears that the conclusion of these authors is not limited to homiotherms. In the calculations of Baron and Polányi, $-\Delta A$ was used instead of $-\Delta F$. The former is the free energy decrease at constant volume and differs from $-\Delta F$ in the same way that $-\Delta V$ differs from $-\Delta H$, viz., the PdV factor is not included.

Utilization of energy

The results of Rubner and Tangl showed that only 15 to 60 per cent of the energy that disappeared from a medium in which bacteria had grown appeared in the cells. Since the work of these investigators, similar results have been obtained for a large number of organisms, some of which are given in table 3.

These figures indicate that during the synthesis of 1 gram of bacterial substance (the equivalent of 4.5 kg-cal.), there is liberated from two to over one hundred and twenty times this quantity of energy. The question naturally arises—of what use is this energy to the bacteria? A few possible expenditures will be taken up in an effort to account for the energy which is apparently at the disposal of the organism.

Energy of synthesis. The compounds synthesized by bacteria and built into their cells include many types found in both plants and animals (9). Carbohydrates of various types have been isolated—polysaccharides, gums, and capsular substances, as well

TABLE 3
Energy of medium transformed into bacterial cells

ORGANISM	MEDIUM	PERCENTAGE OF ENERGY CHANGE REPRESENTED BY BACTERIA
<i>B. pyocyanus</i>	Asparagin	19.0
Lactic acid bacteria.....	Peptone-milk	46.0
<i>Microc. ureae liquefaciens</i>	Urine + urea	0.8-32.0
<i>Bac. aerogenes</i>	Whey + lactose and peptone	6.5-30.0
Acetic acid bacteria.....	Alcohol	0.75
Yeast.....	Wort	22.0-43.0
Mannitol formers.....	Glucose	48.0
Azotobacter.....	Mannitol	5.0-11.0

as reserve materials such as glycogen and granulose. Fatty materials of even greater variety and complexity are known to occur in bacterial cells. These include free fatty acids, glycerides, waxes, sterols, and phosphatides. Simple proteins, as albumin and globulin, have been obtained from bacteria, but the major protein constituents are apparently conjugated proteins such as nucleoproteins.

It is obvious that little can be done toward deciding the question as to the energy requirements for synthesis unless we consider specific reactions. For example, the synthesis of bacterial protoplasm from a mixture of amino acids and polypeptides,

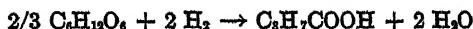
such as beef extract, can be done with much less expenditure of energy than is necessary if the building material consists of glucose and nitrates and other salts. In the latter case energy is required to reduce the nitrate to ammonia and to combine this with decomposition products of glucose to form the various amino acids required for the proteins of the organism. This necessary energy is furnished by an oxidation of part of the glucose, as will be discussed in detail later. Beebe and Buxton (6) have shown that *B. pyocyanus* can synthesize fat on a sugar-free peptone broth, the synthesis of fat from carbohydrates by yeast has long been known, and the work of Lieben (39, 40) and his associates demonstrated that yeast could form carbohydrate material from alcohol, lactic acid and pyruvic acid. A single amino acid can serve as the sole source of carbon and nitrogen for some bacteria, and syntheses of protein from glucose and nitrate, ammonia, or even free nitrogen are not uncommon. An examination of the energy changes involved in these interconversions might be of value in estimating what the energy requirements for synthesis are.

The synthesis of a fatty acid from a carbohydrate necessitates a reduction which requires energy. For example, butyric acid, formed by the reduction of glucose, would yield on complete oxidation 508.2 kg-cal. of free energy, while that given by glucose of an equivalent carbon content (2/3 mole) would be 457.3 kg-cal. The difference, -50.9 kg-cal., represents $-\Delta F$ for the synthesis of 1 mole of butyric acid from 2/3 mole of glucose; the negative sign denotes that energy must be supplied in order that the synthesis might take place. This energy requirement can be arrived at by a consideration of schematic equations which illustrate the reduction. While the following equations are not intended to represent the actual mechanism of the transformations, they serve as a convenient means of presenting the energy relations involved.

(1) Synthesis of butyric acid.



$$-\Delta F^\circ = -113.1 \text{ kg-cal.}$$



$$-\Delta F^\circ = 62.2 \text{ kg-cal.}$$



$$-\Delta F^\circ = 228.6 \text{ kg-cal.}$$



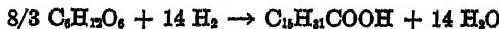
$$-\Delta F^\circ = 177.1 \text{ kg-cal.}$$

The sum of the decrease in free energy of the first two reactions, resulting in the formation of butyric acid, is -50.9 kg-cal. as already noted. This energy could be supplied by the simultaneous oxidation of glucose as in the third reaction. The excess of energy liberated in the complete reaction would be available for other energy-requiring processes or could be liberated as heat. In the foregoing equations, it was assumed that oxygen or other hydrogen acceptors are present that can oxidize any hydrogen liberated. If the hydrogen appears as the free element, the net free energy released by the final complete equation would be less by $56.56n$ kg-cal. where n equals the number of moles of hydrogen set free in the process.

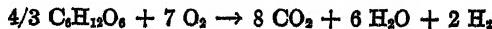
(2) Synthesis of palmitic acid. The synthesis of palmitic acid requires 490 kg-cal. of free energy, as indicated by the following equations:



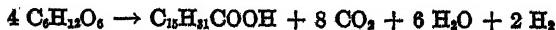
$$-\Delta F^\circ = -791.8 \text{ kg-cal.}$$



$$-\Delta F^\circ = 301.8 \text{ kg-cal.}$$



$$-\Delta F^\circ = 801.4 \text{ kg-cal.}$$



$$-\Delta F^\circ = 311.4 \text{ kg-cal.}$$

If the synthesis is carried out in the presence of oxygen or other hydrogen acceptors, the liberated hydrogen may be oxidized to water and the net decrease in free energy will be greater by 113.1 kg-cal.

(3) Synthesis of amino acids. The energy transfers that accompany the formation of an amino acid from a carbohydrate may liberate or require energy depending upon the individual amino acid, the source of nitrogen, and whether or not free hydrogen is disengaged in the reaction. If the amino acid is relatively simple and ammonia nitrogen is available, there is a release of energy.²

Synthesis of alanine



$$-\Delta H = -11.4 \text{ kg-cal.}$$



$$-\Delta H = -22.6 \text{ kg-cal.}$$



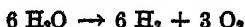
$$-\Delta H = 68.4 \text{ kg-cal.}$$



$$-\Delta H = 34.4 \text{ kg-cal.}$$

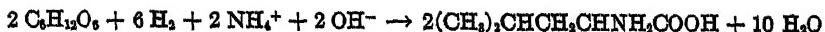
However, energy is required for the synthesis of a higher amino acid, even though the source of nitrogen is already reduced, e.g., ammonia nitrogen. For example, leucine requires 97.5 kg-cal. per mole for its formation from glucose and ammonia. This energy can be furnished by the combustion of glucose, as illustrated with the fatty acids.

Synthesis of leucine

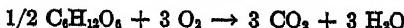


$$-\Delta H = -410.3 \text{ kg-cal.}$$

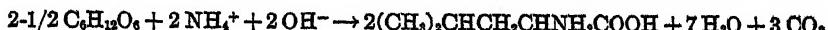
² In the calculation of the energy changes coincident with the formation of amino acids, it was necessary to use $-\Delta H$ as a measure of the changes taking place since free energy data on the amino acids are not available. This measure is to be regarded as a more or less close approximation of the more exact $-\Delta F$.



$$-\Delta H = 215.2 \text{ kg-cal.}$$

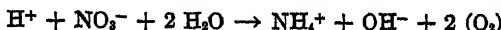


$$-\Delta H = 337.0 \text{ kg-cal.}$$

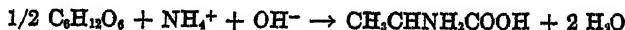


$$-\Delta H = 141.9 \text{ kg-cal.}$$

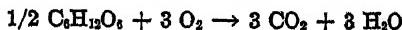
If the source of nitrogen used by the organism is in the form of a nitrate, energy must be expended to reduce this to ammonia nitrogen. In this case the formation of an amino acid as simple as alanine requires 65.9 kg-cal. of energy, which can be supplied by the oxidation of a carbohydrate.



$$-\Delta H = -100.3 \text{ kg-cal.}$$



$$-\Delta H = 34.4 \text{ kg-cal.}$$



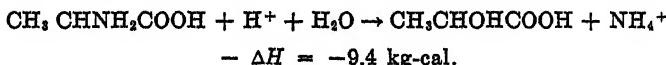
$$-\Delta H = 337.0 \text{ kg-cal.}$$



$$-\Delta H = 271.1 \text{ kg-cal.}$$

There is a third way by which the needed ammonia might be obtained for the synthesis of amino acids, i.e., the hydrolysis of other amino acids. If an organism is grown in a mixture of amino acids (as beef broth, peptone) the energy value of which is very near that of the cell, the assumption is often made that no energy is required to convert the constituents of the medium into cell protoplasm. This is not necessarily the case, since it is more likely that the organism changes the substrate into other forms before using it for synthetic purposes. However, the energy

changes are not of a large order of magnitude since hydrolyses predominate among the reactions.



From a consideration of these reactions it is apparent that energy is necessary to synthesize fat from carbohydrates, and that amino acids may be formed from carbohydrate with or without expenditure of energy depending upon the source of nitrogen, complexity of the acid, and the availability of oxygen or of hydrogen acceptors. Conversely, the production of carbohydrate from fats would liberate energy, etc. If the proper amino acids, fatty acids, and simple sugars are available, the steps to convert these into protein, esters, and polysaccharides involve little or no energy changes (26, 66).

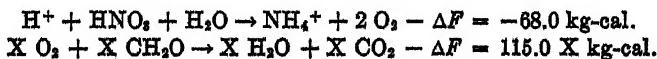
Experimental data that would help to solve the problem of the energy expenditure for syntheses are sadly lacking. Lundin (42), Meyerhof (47), and others have shown that yeast in the presence of oxygen can convert alcohol and lactic acid into glycogen and an "unhydrolyzable" carbohydrate and that these syntheses apparently take place exothermically. Attempts have been made to calculate the energy required for synthesis from the difference between the heat liberated in fermentations by growing and non-growing cells, but the data are too few to draw definite conclusions. From Rubner's (61) data on the heat of fermentation of sucrose by proliferating and non-proliferating yeast cells, it appears that only 4.1 per cent of the energy liberated in the fermentation is stored in the cells. The data of Brown (8) on fermentation of maltose show, however, that 8.8 per cent less heat is liberated by growing than by non-growing yeast cells. In all of these experiments, the substrate used was equivalent to a mixture of amino acids (peptone, beer-wort). Additional experiments of a similar nature with bacteria, in which the substrate is varied, are necessary before a definite answer can be given to the question of how much energy is required for synthetic purposes. Especially desirable for this purpose are accurate data from experiments in which the bacteria synthesize their

protoplasm from simple organic carbon sources as glucose and an inorganic nitrogen source, e.g., ammonia or nitrate nitrogen.

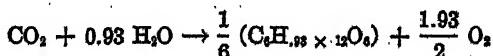
In the foregoing discussion of the energy required for growth it should be pointed out that two types of energy efficiency have been considered. In the theoretical equations, the free energy available to the organism as shown by the energy balances is the maximum useful work given by the reaction, and the efficiency of its use by the organism will depend on the reversibility of the reaction involved, i.e., the presence or absence of simultaneous, irreversible, frictional processes that lead to dissipation, ordinarily as heat, of part of the free energy liberated. However, the data given are concerned with the efficiency of total processes, i.e., the percentage of the maximum total energy available that can be accounted for by the organism in the synthesis of body matter etc. Due to the irreversibility and hence heat losses attendant on these syntheses, the efficiency indicated by the data is fairly low. Burk (11a) has stressed the importance of this distinction of efficiency in a recent paper dealing with a proof of the validity of the second law in a biological process. He emphasizes the point that in a study of the efficiency of a given reaction from a point of view of the energy relations, it is often desirable, where possible, to separate and to correct for any simultaneous, irrelevant processes whose high degree of irreversibility leads to a low value for the observed efficiency. In every process studied, two types of efficiency are present which he has termed "machine efficiency" and "second law efficiency." "Machine efficiency" is defined as the total work done in the process (chemical or otherwise) divided by the total free energy dissipated by the organism in carrying out all of its life processes. The data in table 3 dealing with the percentage recovery, as bacterial dry matter, of the total energy that disappears from the medium, are examples of "machine efficiency." "Second law efficiency" is defined as the total work done divided by the free energy dissipated solely in the reaction studied. Unfortunately it is not always possible to calculate the second law efficiency because of inability to separate the energy relations of the process being studied from those of other simultaneous processes.

Burk, however, has been able to make a complete energy balance sheet for the autotrophic hydrogen oxidizer, *B. pycnoticus*. He showed that: (1) the "machine efficiency" of this organism varies widely with change in experimental conditions and has an average of 28.4 per cent; (2) if proper correction is made for extraneous irreversible processes, the efficiency of the coupled reactions involving the oxidation of hydrogen and the reduction of carbon dioxide by this organism is practically perfect (i.e., about 100 per cent) in its reversibility and independent of physiological experimental conditions. In some other processes considered (including autotrophic methane oxidation, heterotrophic nitrate reduction, photosynthesis of carbon dioxide, muscle contraction) it was not possible to secure evidence for 100 per cent efficiency, presumably due to the lack of sufficient or reliable data to make the necessary corrections.

Burk, however, suggests another possibility that would explain the observed lower efficiency in certain cases, depending on what was termed the "biochemical reversibility principle," a principle which stands in relation to the second law of thermodynamics as the second law does to the first law, viz., as conditioning the convertibility of energy from one form to another. The coupled reaction for the heterotrophic reduction of nitrate can be written:



With perfect reversibility (100 per cent efficiency) X would equal $\frac{\Delta F}{\Delta F} = 0.59$. However, the experimental results indicate that X equals 2.0, which is the number of moles oxygen liberated in the energy-requiring process (corresponding to a "second law efficiency" of about 32 per cent). Similarly with the oxidation of hydrogen and reduction of carbon dioxide, the energy relations indicated that X should equal 0.97, which was also that demanded by the stoichiometry of the energy-requiring equation, viz:



In this case, as already noted, the observed "second law efficiency" was 100 per cent. These two cases suggest that the maximum "second law efficiency" to be expected in isolated coupled reactions is determined not only by the energy relations of the two reactions but also by the stoichiometry of the reactions. This means that the amount of the energy-yielding reaction that takes place is determined not solely by the free energy demanded by the energy-requiring process, but also by the stoichiometry of this latter process. Quoting Burk, "Expressed in more strictly chemical terms, all the incidental compound produced in the free energy-requiring reaction (in the above particular cases, oxygen) must, by virtue of the mechanism chosen by the organism, be consumed in the free energy-yielding reaction." Although these two reactions are the only ones with sufficient data to test this principle, its implications should be kept in mind as a possible explanation of apparent "wastefulness" of free energy by microorganisms. It is hoped that eventually some of the processes of heterotrophic organisms can be investigated in greater detail by this isolation principle proposed by Burk, and that definite information will replace the present rough approximation that reversibility in heterotrophic organisms is between 0 and 50 per cent.

Energy of motion. At first thought one might expect that much of the energy available to the organisms would be used in their motion. Indeed, Rubner attributed differences in energy of maintenance of growing compared with non-proliferating cells to be due to the difference in motility. Calculations, however, indicate that the energy expended in moving is infinitesimal in comparison with that usually available. Angerer (1) has calculated this by an indirect method; by means of Stokes' law, he estimated the rate at which an organism would fall in a medium. The force responsible for this fall is that of gravity (981 dynes = 1 gram). By a comparison of the rate of fall under gravity and the rate of motion, he estimated the force necessary to move the bacteria. The authors have made a direct calculation by use of a formula in hydrodynamics (37) that gives the force necessary to propel an ellipsoid of the prolate spheroid type, a form that many bacteria approach in their morphology, through a medium of known viscosity.

$$F = 6 \pi \mu RV$$

$$R = \frac{8}{3} \left(\frac{abc}{X_0 + \alpha_0 a^2} \right)$$

$$X_0 = abc \int_0^\infty \frac{d\lambda}{\Delta}$$

$$\frac{2 ab^2}{\sqrt{a^2 - b^2}} \ln \left[\frac{a + \sqrt{a^2 - b^2}}{b} \right] \text{ when } b =$$

$$\alpha_0 = abc \int_0^\infty \frac{d\lambda}{(a^2 + \lambda) \Delta}$$

$$= \frac{2 ab^2}{\sqrt{(a^2 - b^2)^3}} \left[\ln \frac{b}{a - \sqrt{a^2 - b^2}} - \frac{\sqrt{a^2 - b^2}}{a} \right] \text{ when } b = c$$

where μ equals viscosity of liquid; V equals velocity of motion; a, b, c equal semi-axes; and R equals effective radius defined in terms of a, b, c, X_0 and α_0 ; X_0 and α_0 are defined³ in terms of the definite integrals in which $\Delta = \sqrt{(a^2 + \lambda)(b^2 + \lambda)(c^2 + \lambda)}$.

Table 4 gives a comparison of the data found by the two methods. The authors' calculations were based on the assumption that the bacteria are prolate spheroids with a density equal to 1.055. Although the agreement between the two methods of calculation is not absolute, the results are of the same order of magnitude and indicate that the energy expended for movement by bacteria is only an extremely small portion of that transformed. If the average energy of maintenance is regarded as about 20 kg-cal. per gram of bacterial nitrogen per day, and if the nitrogen content of the wet cells is taken as approximately 2.5 per cent, the energy of maintenance will be about 500 g-cal. per gram of wet bacteria per day. Of this, less than 0.5 g-cal. are used for

³ It should be noted that α_0 and X_0 are not functions of λ which is merely an integration parameter. Thus it is not necessary to give physical significance to λ . If, however, one writes $\frac{x^2}{a^2 + \lambda} + \frac{y^2}{b^2 + \lambda} + \frac{z^2}{c^2 + \lambda} = 1$, then for given values (x_0, y_0, z_0) of x, y , and z , this equation is a cubic in λ whose three roots are defined as the ellipsoidal coördinates of the point x_0, y_0, z_0 .

motion by the sluggish organisms and 1 to 2 g-cal. by the more speedy types. In these calculations it is assumed that the means of locomotion are 100 per cent efficient. This probably is not the case, but even with an efficiency of only 10 per cent the energy

TABLE 4
Energy required for movement by different bacteria

ORGANISM	VELOCITY	FORCE		WORK DONE PER GRAM BACTERIA PER DAY	
		Angerer	Authors	Angerer	Authors
	centimeter per second	grams $\times 10^4$	grams $\times 10^{11}$	gram-calories	gram-calories
<i>V. cholerae</i> ($2.0\mu \times 0.4\mu$).....	0.003	8.6	2.06	1.63	0.71
<i>B. typhosus</i> ($2.1\mu \times 0.7\mu$).....	0.0018	5.4*	1.70	0.22*	0.11
<i>B. subtilis</i> ($2.1\mu \times 1.0\mu$).....	0.001	3.0	1.18	0.03	0.02

*This figure was recalculated from Angerer's data, as his calculation contained a numerical error.

TABLE 5
Relationship between oxygen consumption and motility of bacteria
Wohlfeil

ORGANISM	MOTILITY	OXYGEN CONSUMPTION PER HOUR PER 10^6 ORGANISMS cc. $\times 10^7$
Experiment 1:		
<i>Bac. dysenteriae</i> (Shiga).....	-	6.80
<i>Bac. enteritidis</i> (Gärtner).....	\pm	6.74
<i>B. coli commune</i>	\pm	8.41
<i>Bac. typhosus</i>	+	8.44
<i>Bac. paratyphosus B.</i>	+++	9.10
<i>B. faecalis alcaligenes</i>	++++	11.54
Experiment 2— <i>B. coli commune</i> :		
Strain 1.....		5.40
Strain 2.....		4.06
Strain 3.....		3.87
Strain 4.....		3.35

expended in motion is an inconsiderable share of the total available.

Of interest in this connection are data published recently by Wohlfeil (73), who measured the oxygen consumption of bacteria

which are closely related but which exhibit varying degrees of motility. His data are summarized in table 5.

From the data of the second experiment in which four strains of *B. coli* were used, Wohlfeil decided that the oxygen consumption is fairly constant among members of the same species, therefore the difference noted among the organisms of experiment 1 constituted real and not apparent differences. He assumed that the difference noted in the oxygen uptake by *Bact. dysenteriae* and *Bac. typhosus* is due to the motility of the latter with a consequent higher energy requirement. He converted the difference into gram-calories by use of the relationship 1 cc. O₂ = 4.67 g-cal., and thus calculated the energy required for motion by *Bac. typhosus* to be 2.15×10^{-19} g-cal. per second per bacterium. He noted that this result agrees remarkably well with the figure calculated by Angerer for the energy requirements of this organism for motion, viz., 2.80×10^{-19} g-cal. per second. Unfortunately there is an error in the calculation of Angerer for this organism. The correct value for the energy requirements for motion by *Bac. typhosus* calculated according to Angerer's method is 23.0×10^{-19} g-cal. per second per bacterium. Calculated according to the formula given on page 447 this is reduced to 7.2×10^{-19} g-cal. per second per bacterium. However, the difference between the experimental value of Wohlfeil and these calculated values may be due to the fact that not all of the organisms are in motion, as is assumed in the calculations. Wohlfeil mentions that random fields of *Bac. typhosus* showed only one out of five or even one out of ten of the organisms in motion.

A more severe criticism of Wohlfeil's data is that of the statistician. The mean of the four strains of *B. coli* is 4.7×10^{-7} cc. oxygen per hour per 10^8 bacteria. The standard deviation of the individual values is 0.754×10^{-7} or about 18 per cent of the mean. While it would be highly desirable to have more than four samples for the calculation of these statistics, the data available suggest that differences from the mean of 36 per cent or less (twice the standard deviation) may well arise from chance. *Bac. typhosus* had an oxygen consumption only 24 per cent higher than that observed for *Bac. dysenteriae*. In view of the variations noted

among the strains of *B. coli*, this difference cannot be considered significant as it stands. The variation among the strains of *B. coli* might have arisen from differences in motion by these strains since Wohlfeil reports their motility as \pm , i.e., they are only partly motile. In spite of these objections, the data of Wohlfeil are not without value; it appears that there is a high positive correlation between motility and oxygen consumption. Further work, in which a number of strains of each species are used in order to control the individual differences, might yield extremely interesting and more conclusive results.

Energy for increase of surface. When the question of the energy expended in increasing the surface of bacteria during growth is considered, the lack of data on the interfacial tension between the cells and the medium requires the use of indirect methods that may or may not yield valid conclusions. A bacterium 2μ in length and 1μ in diameter, considered as a cylinder, would have a surface of 7.85×10^{-8} sq. cm. and a volume of 1.57×10^{-12} cc. Thus it would require 6.2×10^{11} wet bacteria to weigh 1 gram (density = 1.055). This 1 gram of bacteria would have a surface equal to 4.88×10^4 sq. cm. Since 1 calorie = 4.182×10^7 ergs, the work of adhesion between the cell and the medium (disregarding the small amount of surface present in the inoculum) would have to be $\frac{4.182 \times 10^7}{4.88 \times 10^4} = 857$ ergs per sq. cm. in order to use 1 g-cal.

equivalent of energy in the production of the surface of 1 gram of bacteria of the above dimensions. In like manner, the work of adhesion necessary to produce the surface represented by 1 gram of wet bacteria of the dimensions 1.0μ by 0.5μ would be 441 ergs per sq. cm. if 1 g-cal. equivalent of energy were used in the process. These values are much higher than those found by Harkins (25) for the work of adhesion between organic liquids and water. Tangl (65) reports that the total energy expended in the production of 1 gram of bacteria is 2000 to 4000 g-cal., consequently on the basis of the foregoing data, it appears that the work done in increasing the surface must be small in comparison with this total. It should not be overlooked that the work of adhesion between the cell membrane-medium interface might be markedly

different from the organic liquid-water interfaces studied by Harkins. However, there is some indirect evidence in support of the view that the interfacial tension between the cell and the medium is rather low. Davis (16) found that the effect of various cations on the size of oil drops (oleic acid) in water paralleled their effect on the growth of *B. coli*. He suggests that the surface tension of bacteria is essentially that of a water-oil interface. If this is true, then it can be concluded that only a fraction of a g-cal. is necessary to increase the (external) surface of 1 gram of bacteria (in contrast to the several thousand calories available). Support of this low value for the interfacial tension between bacterium and water is offered by the work of Mudd and Mudd (49). These workers studied the behavior of bacteria in water-oil interfaces. Their data suggest that most bacteria act as a semi-solid gel with polar groups oriented in the surface so that the interfacial tension between the cell and water is rather low. Acid-fast organisms, however, have a smaller interfacial tension toward the oil phase than toward water. The calculations of the surface formed in the production of 1 gram of bacteria involved the assumption that this surface was smooth. If the cell membrane is sponge-like in character, as is a silica gel, then the surface area would be greatly increased. In spite of these assumptions, it seems likely that the work done in the production of the external surface of bacteria uses only a small part of the total energy available.

Other expenditures of energy. From the foregoing discussion, it must be concluded that the available information concerning the manner of expenditure of energy by the cell is of a rather negative character. It appears that only a small part of the energy that is transformed can be accounted for; the remainder of the energy finally appears as heat, but it is not known whether this heat arises directly from the chemical energy liberated or is the result of work done by it. Warburg (71) has determined the quotient $\frac{\text{work done}}{\text{maximal work available}}$ for a number of cells, and has found that except in the case of the active muscle this quotient was extremely small. He attributes this to the fact that work

is being done that is not apparent. Examples of this type of work would be: retention of phase boundaries, creation of internal surfaces, prevention of diffusion, and osmotic work done in transportation of substances from a region of low concentration to a region of higher. Nathanson (50) has suggested that bioelectric potentials originate in cells due to differences in concentration of ions on either side of a membrane or through selective adsorption. This potential is then capable of decomposing water, and by immediate depolarization a supply of nascent oxygen is maintained for cellular oxidation. This electric force is also the means of supplying the energy for secretion and adsorption. If this hypothesis is correct, then part of the energy unaccounted for is used in the production and maintenance of unequal concentrations on either side of the membrane in order to produce the necessary potential. In this connection it must be remembered that the change of free energy of all processes at the equilibrium point is zero. All the reactions taking place in the cell are moving toward equilibrium, hence work must be done continually to keep these equilibrium points from being reached so that the metabolic processes can continue.

Giaja (21) has reported that the "fundamental biologic energy" of yeast—that is, the energy necessary to maintain the cells when no external work is done—is much smaller than that liberated by yeast in the presence of sugar. He regards the energy liberated when non-proliferating cells are placed in sugar solutions as due to uncontrolled activity of the enzymes present in the cell. A purpose for this apparent excess liberation of energy was suggested by Brown (8). He found that yeast required only 2.2 hours to ferment its own weight of maltose, and calculated that the metabolism of yeast must be about seventy times that of man. If the heat liberated by the yeast in its metabolism were not dissipated into the surrounding medium, the temperature of the cell would be raised 75.5°C. per hour at 30°C. Brown attributed this large heat liberation to the fact that in the natural habitat of the organism the heat losses are so great that such liberation of heat is required to keep the cell at the optimum temperature. When the yeast is placed in the artificial environ-

ment of the laboratory medium, this excess energy is no longer needed, but is liberated because of the lack of control of the cell over its enzymatic activities.

These conclusions are based on the assumption that the cell liberates its heat under strictly adiabatic conditions. However, unless the organism is fairly well insulated, it appears that even the high rate of heat production noted by Brown would be insufficient to maintain its temperature much above that of the surrounding soil, fruit, or other natural substrate. An approximation of the rate of heat production necessary to keep a colony of yeast cells at a temperature higher than that of its environment can be made by the following formula (30). In the application the colony of yeast is considered as a sphere surrounded by a spherical coating of soil.

$$E = \frac{4\pi k (T_1 - T_2) r_1 r_2}{r_1 - r_2}$$

E = rate of heat production in gram-calories per second necessary to produce a temperature difference between surfaces of the two spheres,

k_1 = heat conductivity of soil (dry soil = 0.00033),

r_1 = radius in centimeters of outer sphere (soil + colony),

r_2 = radius in centimeters of inner sphere (colony),

$T_1 - T_2$ = difference in temperature of the surfaces of the inner and outer spheres.

Table 6 gives a summary of calculations made for colonies of various radii surrounded by a layer of soil of different thicknesses.

These figures show that only large colonies with thick layers of insulation are able to maintain a temperature above that of the environment. Small colonies, because of the large surface per unit volume, are unable to retain the heat liberated in the metabolism of the cells. The formula shows that for a given insulating substance of heat conductivity k , the rate of heat production necessary to keep the temperature of the surface of the inner sphere 1°C. higher than that of the outer sphere approaches a minimum equal to $4\pi kr_2$ as r_1 is increased. The calculations show that for a colony 1 mm. in radius the potential difference in

temperature increases from 0.04°C. to 0.19°C. as the layer of soil is increased from 0.25 mm. to 99.0 mm. Also the temperature difference changes very little with further increase in the thickness of the soil layer. It follows that within certain limits (from two to ten times the radius of the inner sphere) it makes little difference as to what is assumed for the thickness of the soil layer. In the above calculations the figure for the heat conductivity of dry soil is used; in a moist soil the conductivity would be higher

TABLE 6
Potential temperature differences between environment and yeast colonies of varying size

RADIUS OF COLONY (r_2) millimeters	RADIUS OF COLONY AND SOIL (r_1) millimeters	RATE OF HEAT PRODUCTION		TEMPERATURE DIFFERENCE °C.
		Required for difference of 1°C. gram-calories per second $\times 10^4$	Available* gram-calories per second $\times 10^4$	
1	1.25	20.75	0.786	0.04
1	2.0	8.30	0.786	0.10
1	10.0	5.20	0.786	0.15
1	100.0	4.20	0.786	0.19
5	10.0	41.50	98.20	2.36
5	100.0	21.90	98.20	4.48
10	20.0	83.0	786.0	9.46
10	100.0	43.7	786.0	18.00

* Calculated on the assumption that yeast ferments its own weight of maltose in 2.2 hours.

and the potential rise in temperature of the colony correspondingly smaller. In a well-insulated environment or with large masses of cells the rate of heat production by microorganisms is sufficiently high to cause a rise in temperature in the immediate vicinity, as is noted in the biological heating of manure. However, it appears unlikely that microorganisms in general in their natural habitats produce heat at a rate sufficient to maintain an optimum temperature above that of their surroundings.

II. THE APPLICATION OF ENERGETICS TO FERMENTATIVE PROCESSES

Alcoholic fermentation

The energetics of this fermentation have been more completely investigated than any other because of its industrial importance.

Rubner (58, 59) studied the total heat produced, the heat produced by the action of zymase and invertase and determined the influence of alcohol, nitrogen nutrition, and other factors on the energy changes. He found that the heat liberated by zymase was only a small part of the total liberated, and used this as an argument for the theory that most of the fermentation resulted from cells *per se*. A carbon balance indicated that 1 gram of sucrose should liberate about 159.4 g-cal.,⁴ whereas that actually measured in the calorimeter was 149.9 g-cal. per gram of sucrose. The calculated value for glucose was 147.6 g-cal.⁴ per gram and the measured value was 133.3 g-cal. per gram. The concentration of sugar and of yeast did not affect these values but only the

TABLE 7
Heat production by different species
Rubner

SPECIES	HEAT PRODUCTION PER DAY PER KILO WEIGHT	HEAT PRODUCTION PER DAY PER SQUARE METER OF BODY SURFACE
	kilogram-calories	kilogram-calories
Man.....	30.0	1042
Dog.....	45.9	1039
Mouse.....	210.0	1039
Yeast.....	1163 (30°C.)	0.84-1.2
Yeast.....	1173 (38°C.)	

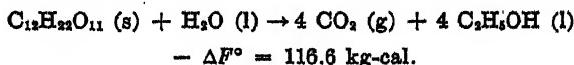
rate of heat production; the presence of alcohol resulted in a decrease of the total heat production. Table 7 gives a comparison of the heat produced by different species, although Rubner pointed out that little agreement should be expected between species with different physiological and anatomical characteristics.

Fulmer and Leifson (19) have made a noteworthy contribution to the study of the free energy changes occurring in the formation of alcohol from sucrose. These authors also outlined in detail the methods suitable for calculation of the change in $-\Delta F$ with changes in concentrations of the reactants and resultants and

⁴ Calculated from more recent thermal data.

stressed the importance of considering these energy changes when dealing with biological transformations.

From the equation



they calculated the change in $-\Delta F$ when 0.3 mole of sucrose is fermented for various initial concentrations of sucrose and ethyl alcohol and for a constant pressure of 0.0003 atm. of carbon dioxide (air). The data in table 8 are taken from their paper.

These data show that the maximum difference between the free energy decrease for concentrations of sucrose, ethyl alcohol, and

TABLE 8

Average values of $(\Delta F - \Delta F^\circ)^$ per mole of sucrose fermented when $-\Delta M = 0.3$ mole*

Fulmer and Leifson

INITIAL CONCENTRATION OF SUCROSE	INITIAL CONCENTRATION OF ETHYL ALCOHOL		
	$2.1 \times 10^{-4}M$	$2.1 \times 10^{-3}M$	$2.1 \times 10^{-2}M$
<i>M</i> †	<i>kilogram-calories</i>	<i>kilogram-calories</i>	<i>kilogram-calories</i>
1.00	-27.42	-24.65	-23.02
0.70	-26.52	-23.75	-22.61
0.50	-24.95	-23.36	-22.08

* $-\Delta F$ = decrease in free energy under conditions indicated in table.

$-\Delta F^\circ$ = decrease in free energy in standard states = 116.6 kilogram-calories.

† *M* = molality.

carbon dioxide likely to be present in a fermentation and the $-\Delta F^\circ$ (standard states) is about 27 kg-cal., or an increase of 23 per cent. The authors conclude, "Considering the fact that the equation assumed for the fermentation does not represent accurately the products formed by biological action, the corrections from the standard state in this case are not of relatively great importance. However, it is worth while, in each instance, in the use of free energy changes of biological processes to analyze the situation along the lines presented above."

Lactic acid fermentation

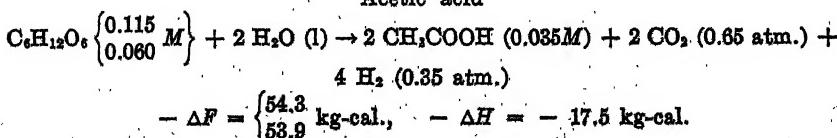
Rubner (60) studied the heat produced by this fermentation both in pure culture and in the spontaneous souring of milk.

Experiments made on the souring of milk showed that only 25 to 45 per cent of the observed heat production could be accounted for by the theoretical equation for the decomposition of lactose into lactic acid. Using sterilized milk inoculated with an "organism producing dextro-lactic acid" he found only 23 per cent of the observed heat could be accounted for by the acid produced. He suggests that other vital processes yielding heat caused these discrepancies, and believes that for this reason heat production is a better method for comparing the metabolism of these organisms than is lactic acid production.

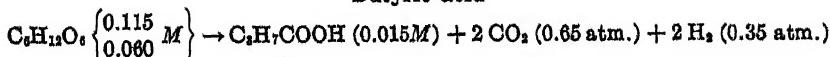
Acetone-butyl alcohol fermentation

A study of the mechanism of this fermentation made in this laboratory (31) has indicated certain theoretical equations for the break-down of carbohydrates into the solvents and acids formed in the fermentation. From data on the fermentation of glucose it was found that the concentrations of acetic and butyric acids varied so little from the tenth to the thirty-second hour that the concentrations may be treated as constant during this period. Also 40 per cent of the glucose disappeared during this time, so that the free energy changes of this period may be taken as fairly representative of the entire fermentation. The free energy equations for the production of the acids showed that the change in the glucose concentration did not materially affect the total decrease in free energy, hence the average value of the glucose was used to calculate the free energy changes involved in the formation of solvents. In the following equations each fermentation product is referred to an individual molecule of glucose for purposes of calculation. In the actual fermentation these products arise undoubtedly from a common intermediate compound rather than each from a discrete glucose molecule.

Acetic acid

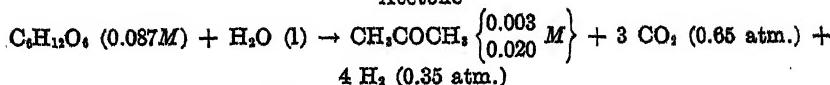


Butyric acid



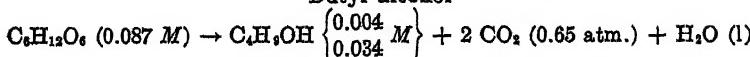
$$-\Delta F = \begin{cases} 69.0 \\ 68.6 \end{cases} \text{ kg-cal.}, \quad -\Delta H = 17.2 \text{ kg-cal.}$$

Acetone



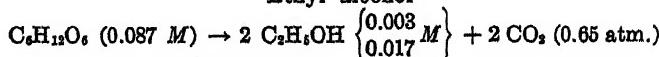
$$-\Delta F = \begin{cases} 49.5 \\ 48.4 \end{cases} \text{ kg-cal.}, \quad -\Delta H = -26.4 \text{ kg-cal.}$$

Butyl alcohol



$$-\Delta F = \begin{cases} 70.7 \\ 69.4 \end{cases} \text{ kg-cal.}, \quad -\Delta H = 35.0 \text{ kg-cal.}$$

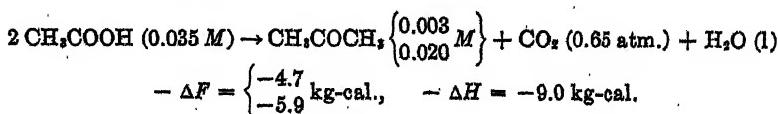
Ethyl alcohol



$$-\Delta F = \begin{cases} 58.7 \\ 57.6 \end{cases} \text{ kg-cal.}, \quad -\Delta H = 18.1 \text{ kg-cal.}$$

From these data it was calculated that from the tenth to the thirty-second hour 0.055 moles of glucose were decomposed with a free energy decrease of 3.34 kg-cal., or $-\Delta F = 60.8$ kg-cal. per mole of glucose fermented. Since the standard free energy of the complete oxidation of glucose is -685.8 kg-cal. per mole, it is seen that the anaerobic decomposition by this organism liberates less than one-tenth of the "potential" free energy. The production of acetic acid and acetone from glucose are examples of reactions in which $-\Delta F$ and $-\Delta H$ have opposite signs.

Of interest is the consideration of two of the proposed steps in the mechanism. Acetone is thought to be formed from acetic acid. As can be seen from the following equation, this reaction has a positive ΔF , hence energy from another reaction would be necessary to cause it to take place. This necessary energy could be obtained from that liberated in the formation of the acetic acid from glucose.

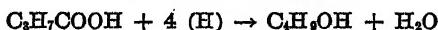


$$-\Delta F = \begin{cases} -4.7 \\ -5.9 \end{cases} \text{ kg-cal.}, \quad -\Delta H = -9.0 \text{ kg-cal.}$$

The reduction of butyric acid to butyl alcohol may require nascent hydrogen. Using the figure given by Lewis for the standard free energy of atomic hydrogen, it appears that the production of butyl alcohol also involves a coupled reaction.



$$-\Delta F^\circ = -88.9 \text{ kg-cal.}$$



$$-\Delta F^\circ = 169.0 \text{ kg-cal.}$$

The first reaction, which requires large quantities of energy to cause it to take place, could obtain this energy from that liberated in the second reaction. In all of these reactions, it is assumed that the concentrations of the reactants and resultants are identical with those of the circumambient liquid. This condition in all probability does not obtain, since the cell apparently alters the concentrations at its active surfaces by performing osmotic work. If the concentrations are changed, the free energy liberated likewise will be changed. However, until more definite information is available, calculations of this nature must serve as first approximations of the free energy change.

Other bacterial fermentations

As pointed out in Part I, if the products of a fermentation are accurately determined, it is possible by an application of thermodynamical data to calculate the heat of reaction and free energy liberated as a result of the fermentation. The energetics of a number of fermentations given in the literature have been calculated by this method. In choosing the data two criteria have been used: (1) the carbon of the products must equal at least 95 per cent of the carbon of the carbohydrate decomposed; (2) the oxidation reactions must balance the reduction reactions at least to within 5 per cent. If the original data fulfilled these conditions they were then adjusted so that the oxidations and reductions were exactly equal: this was most conveniently done by correcting the carbon dioxide or hydrogen figure. After this adjustment, a carbon balance was made by correcting the figure for the carbohydrate fermented so that its carbon was equal to that present in the products. This corrected figure was then com-

pared with the original "carbohydrate fermented" and, if it was within 5 per cent of the latter, the data were judged to be sufficiently accurate for our purpose. These adjustments are necessary since small differences in carbon or oxidation-reduction balances will cause a large difference in the calculated value of the energy liberated. Finally the water that took part in the reaction was calculated by making an oxygen or hydrogen balance, and the entire data were put on the basis of 1000 millimoles (1 mole) of carbohydrate fermented in order to facilitate subsequent calculations.

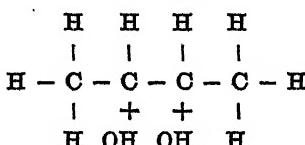
In calculating the standard free energy of the reaction, it was necessary to estimate the free energy of formation of propionic acid, 2,3-butylene glycol, and arabinose, since these data have not been determined. The value for propionic acid was taken to be the average of those for butyric and acetic acids, since Parks and Kelley (54) have shown that the introduction of a CH₃ group in a homologous series causes little change in the value of the free energy of formation. The values for the other compounds were calculated by the "atomic entropy" method of Parks and Kelley as described by Burk (12). Dr. Kelley kindly furnished us with constants based on specific heat measurements of a number of organic compounds down to a temperature of 15°K. These constants are not the same as those given by Burk since the latter are based on earlier, less accurate measurements. It was also necessary to calculate the heat of combustion of 2,3-butylene glycol. This was done by the method of Kharasch (32).

As an example of the use of these methods the calculations for 2,3-butylene glycol are given in detail.

(1). *The heat of combustion of an organic compound.* Kharasch has conceived the oxidation of organic compounds as a process in which energy is released when electrons are displaced from orbits occupied in the methane type of molecules to orbits characteristic of the carbon dioxide type. This change liberates energy equal to 26.05 kg-cal. per electron per mole. In the case of molecules that have substituted groups, the orbits are already partially displaced; hence it is necessary to apply a correction

factor to the general formula, $Q = 26.05 \times N$, where Q = heat of combustion in kg-cal., and N = number of electrons displaced per mole. These correction factors have been worked out by Kharasch and his collaborators, who have shown that the calculated values of the heats of combustion are accurate to within 1 per cent of the experimentally determined values.

From the structural formula of 2,3-butylene glycol the number of electrons displaced is determined (33).



Each hydrogen is positive, hence would be connected with a minus sign to carbon. Carbon to carbon is considered as one negative bond. The oxygens are negative, therefore the connection to carbon is positive. The total number of electrons displaced is the sum of the negative bonds (11) times 2. Kharasch gives the following formula for a polyhydroxy alcohol,

$$Q = 26.05 \times N + 13j + 6.5k + 3.5l,$$

where j equals the number of primary alcohol groups, k equals the number of secondary alcohol groups, and l equals the tertiary alcohol groups. In the case of 2,3-butylene glycol, N equals 22, j and l equal 0, and k equals 2. Therefore,

$$\begin{aligned}
 Q &= 26.05 \times 22 + 2 \times 6.5 \\
 &= 586.1 \text{ kg-cal.}
 \end{aligned}$$

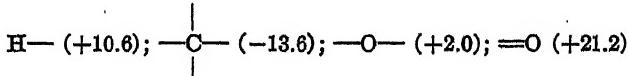
The heat of formation is calculated from the formula,

$$-\Delta H = -Q + 94.38 C + 68.38 H;$$

where Q equals the heat of combustion of the compound, C equals the number of carbon atoms in the compound, and H equals the number of hydrogen moles in the compound. Therefore,

$$\begin{aligned}
 -\Delta H &= -586.1 + 94.38 \times (4) + 68.38 \times (5) \\
 &= 133.8 \text{ kg-cal.}
 \end{aligned}$$

(2). *The free energy of formation of an organic compound.* In order to calculate the free energy of formation it is necessary to know the entropy at 25°C. This can be calculated from the structural formula in a manner similar to the calculation of molecular refraction, volume, etc. The necessary constants were furnished to the authors by Dr. Kelley, who states "For the liquid, aliphatic, saturated alcohols (including polyalcohols), acids and ketones, the following values for 298°K. satisfactorily reproduce the entropies of all the compounds on which we have data:



For each secondary hydroxyl group three units should be subtracted from the value calculated from these constants (based on one result). These constants have little theoretical meaning, but are obtained empirically by solving simultaneous composition equations. Consequently, I believe you can strengthen your case by obtaining the values you desire by comparison, when possible, with similar compounds which have been studied."⁵

This last statement can be illustrated by the calculation used for solid arabinose. Glucose has an entropy value of 50.5 units at 25°C. The data on solid ethylene glycol and glycerol when compared with the figures for erythritol, mannitol, and dulcitol indicate that each CHOH group (in the solid state) is equivalent to 6 or 7 units. Hence the entropy of arabinose was calculated by subtracting 6.5 units from that of glucose; from the value obtained (44.0 m.u.) the free energy of formation of solid arabinose can be calculated. The atomic entropy of 2,3-butylene glycol was calculated:

$$\begin{aligned} 4 \times (-13.6) &= -54.4 \text{ for } 4 \text{ carbon atoms} \\ 10 \times (-10.6) &= 106.0 \text{ for } 10 \text{ hydrogen atoms} \\ 2 \times (-2.0) &= 4.0 \text{ for } 2 \text{ oxygen atoms} \\ 2 \times (-3.0) &= -6.0 \text{ for } 2 \text{ CHOH groups} \\ \hline &= 49.6 \text{ m.u.} \end{aligned}$$

⁵ Private communication to the authors.

This value may be somewhat in error since the correction for the secondary hydroxyl group is based on only one case, viz., the difference between propyl and isopropyl alcohol. From this value for the entropy of 2,3-butylene glycol, the decrease of entropy when this compound is formed from the elements can be calculated if the entropies of the elements are available. Parks, Kelley, and Huffman (55) give the following values for the entropies of the necessary elements: C equals 1.3 E.U. per gram-atom; H equals 14.8,⁴ and O equals 24.5. For $\text{CH}_3(\text{CHOH})_2\text{CH}_3$, the calculation is:

$$\begin{aligned}
 4 \times 1.3 &= 5.2 \\
 10 \times 14.8 &= 148.0 \\
 2 \times 24.5 &= 49.0 \\
 \text{Sum of entropies of elements} &= 202.2 \\
 \text{Entropy of 2,3-butylene glycol} &= 49.6 \\
 - \Delta S_{298} \text{ of formation} &= \underline{\underline{152.6 \text{ E.U.}}}
 \end{aligned}$$

From the relationship $\Delta F = \Delta H - T\Delta S$ the free energy of formation is readily obtained.

$$\begin{aligned}
 \Delta F^{\circ}_{298} &= -133.3 + 298 \times 0.1526 \\
 &= -87.8 \text{ kg-cal.}
 \end{aligned}$$

These calculated values are probably correct to within 2 to 3 per cent. The data for the heats of combustion for the other compounds are those given in the International Critical Tables and by Kharasch; the $-\Delta F^{\circ}$ values are those of Parks, Kelley, and Hoffman (55).

⁴ The value for hydrogen that is now accepted is that of Giauque (J. Am. Chem. Soc. 52, 4816 (1930)), who reports that this value should be 31.28 units per mole instead of 2×14.8 as formerly used. However, since the free energy values of all the other compounds considered in this paper are based on the old value for hydrogen, it was decided to use 14.8 per atom in the calculation of the free energy of formation of 2,3-butylene glycol. In any case the effect of changing the entropy of hydrogen will affect only those reactions in which elemental hydrogen enters as a reactant or resultant. Moreover, the revision necessary for the free energy values of organic compounds, in light of the new value for the entropy of hydrogen, will be of too small an order to affect calculations presented in this paper. Thus, in the case of glucose, the change is less than 1 per cent and would be much the same for similar organic compounds.

The results are given in tables 9, 10, 11, 12, and 13. Table 9 shows the details of the method used in the calculations of energy balances of two fermentations. In the remaining tables only the summaries are given. *B. lactis aerogenes* (table 10) liberates very little heat—3.1 to 5.4 kg-cal. in the fermentation of glycerol—but the maximum free energy available for use is higher. The

TABLE 9
Detailed calculations of energy balances of two fermentations

SUBSTRATE AND ORGANISM.....	<i>B. coli</i> ON GLYCEROL			<i>B. coli</i> ON GLUCOSE		
	FERMENTATION BALANCE.....	$-\Delta H \times$ mole fraction	$-\Delta F^\circ \times$ mole fraction	FERMENTATION BALANCE.....	$-\Delta H \times$ mole fraction	$-\Delta F^\circ \times$ mole fraction
	milli- moles	kilogram- calories	kilogram- calories	milli- moles	kilogram- calories	kilogram- calories
Compound fermented.....	1000	-159.7	-115.7	1000	-302.5	-219.0
Products formed:						
Formic acid.....	125	12.50	10.65	163	16.30	13.89
Acetic acid.....	90	10.49	8.59	480	55.92	45.79
Lactic acid.....	128	20.75	16.15	916	148.65	115.60
Succinic acid.....	31	6.99	5.60	87	19.59	15.70
Ethyl alcohol.....	688	45.31	29.72	536	35.32	23.16
2,3-Butylene glycol.....	99	13.20	8.70	—	—	—
Hydrogen.....	313	—	—	596	—	—
Carbon dioxide.....	415	39.17	39.12	709	66.91	66.83
Water.....	346	23.66	19.57	-336	-22.98	-19.00
$\Sigma -\Delta H$ products \times mole fraction of each.....		172.1			319.7	
$-\Delta H$ of fermentation per mole substrate.....		12.4			17.2	
$\Sigma -\Delta F^\circ$ products \times mole fraction of each.....			138.1			262.0
$-\Delta F^\circ$ of fermentation of 1 mole of substrate.....			22.4			43.0

data for *B. coli* in the same table indicate that, while the heats of reaction in the decomposition of glycerol and of glucose are much the same, the free energy liberated is much greater in the fermentation of glucose. This is due to qualitative and quantitative differences in the products formed. No definite conclusions can be reached as to the constancy of this difference since it may be only a question of variation in strains. However, fermentations

of glycerol by two strains of *B. coli*, one reported by Harden and Norris in 1912, the other by Braack in 1929, give similar results for the energy exchange.

Table 11 presents a study of the energetics of different strains of the propionic acid bacteria on glucose. The data for strains 4,

TABLE 10
Energy balance of coli-aerogenes bacteria

ORGANISM	<i>B. lactis aerogenes</i>			<i>B. coli</i>	
	(7)	(7)	(7)	(24)*	(22)
SUBSTRATE	GLYCEROL	GLYCEROL	GLYCEROL	GLYCEROL	GLUCOSE
	millimoles	millimoles	millimoles	millimoles	millimoles
Total fermented.....	1000	1000	1000	1000	1000
Products formed:					
Formic acid.....	130	146	145	125	163
Acetic acid.....	75	57	31	90	480
Lactic acid.....	32	10	168	128	916
Succinic acid.....	65	54	92	31	87
Ethyl alcohol.....	862	886	756	688	536
Hydrogen.....	751	872	543	313	596
Carbon dioxide.....	640	722	409	415	799
Water.....	92	18	202	346	336†
	<i>kilogram-calories</i>	<i>kilogram-calories</i>	<i>kilogram-calories</i>	<i>kilogram-calories</i>	<i>kilogram-calories</i>
- Δ <i>H</i> of substrate fermented.....	+159.7	+159.7	+159.7	+159.7	+302.5
Σ - Δ <i>H</i> products × mole fraction of each.....	165.1	162.8	168.3	172.1	319.7
- Δ <i>H</i> of fermentation per mole substrate.....	5.4	3.1	8.6	12.4	17.2
- Δ <i>F°</i> of substrate fermented.....	115.71	115.71	115.71	115.71	219.0
Σ - Δ <i>F°</i> products × mole fraction of each.....	136.9	136.2	135.8	138.1	262.0
- Δ <i>F°</i> of fermentation per mole substrate.....	21.2	20.5	20.1	22.4	43.0

* 99 millimoles of 2,3-butylene glycol formed in this fermentation.

† This water reacts with the glucose to form the products.

14, and 12 are taken from the paper by Foote, Fred, and Peterson (18), while those for the other two come from the work of Van Neil (67). In spite of differences in the relative distribution of the acids formed by the various strains, the energy liberated is

practically the same in all cases. The values of $-\Delta H$ and $-\Delta F^\circ$ for this fermentation are much higher than those of any of the others studied.

The data given in table 12 were collected in an effort to ascertain the differences in the energy liberated by the metabolism of the same organism on a number of carbohydrates. The species

TABLE 11

Energy balance of the fermentation of glucose by the propionic acid formers

PROPIONIC ACID FORMERS	STRAIN 4	STRAIN 14	STRAIN 12*	STRAIN 6	STRAIN 22
REFERENCE.....	(18)	(18)	(18)	(67)	(67)
	millimoles	millimoles	millimoles	millimoles	millimoles
Glucose fermented.....	1000	1000	1000	1000	1000
Products formed:					
Acetic acid.....	676	717	645	539	651
Propionic acid.....	1015	1170	1293	1295	1256
Succinic acid.....	310	134	38	112	87
Carbon dioxide.....	353	518	628	591	584
Water.....	672	654	665	702	670
	kilogram-calories	kilogram-calories	kilogram-calories	kilogram-calories	kilogram-calories
$-\Delta H$ of glucose fermented.....	302.5	302.5	302.5	302.5	302.5
$\Sigma -\Delta H$ products \times mole fraction of each.....	350.8	349.1	347.9	348.6	348.5
$-\Delta H$ of fermentation per mole glucose.....	48.3	46.6	45.4	46.1	46.0
$-\Delta F^\circ$ of glucose fermented.....	219.0	219.0	219.0	219.0	219.0
$\Sigma -\Delta F^\circ$ products \times mole fraction of each.....	288.5	290.0	290.6	290.4	290.4
$-\Delta F^\circ$ of fermentation per mole glucose.....	69.5	71.0	71.6	71.4	71.4

* 17 moles lactic acid formed in this fermentation.

chosen was *Lactobacillus pentioaceticus*—the so-called mannitol former. It is to be noticed that the energy liberated in the fermentation of glucose is practically the same as that set free from galactose, but the fermentation of fructose, in which mannitol is formed by reduction of the sugar, results in a large decrease in the energy liberated. The fermentation of maltose gives an energy yield very similar to that of glucose. The energy liberated in the

sucrose fermentation is between that of glucose and fructose, as is to be expected.

Table 13 gives the results of a similar study, using the industrial acetone-butyl alcohol organism, *Cl. acetobutylicum*. These data, like those in table 12, show that as the source of carbon is varied,

TABLE 12
Energy balance of the fermentation of sugars by the mannitol formers

REFERENCE.....	(64)	(64)	(20)	(20)	(68)
CARBOHYDRATE.....	GLUCOSE	GALACTOSE	FRUCTOSE	MALTOSE*	SUCROSE
	millimoles	millimoles	millimoles	millimoles	millimoles
Total fermented.....	1000	1000	1000	1000	1000
Products formed:					
Acetic acid.....	99	49	401	314	187
Lactic acid.....	916	873	309	642	689
Succinic acid.....	—	—	—	9	15
Ethyl alcohol.....	1018	1095	—	934	670
Mannitol.....	—	—	657	—	230
Carbon dioxide.....	1018	1095	328	1017	778
Water.....	—	—	-328†	-83†	-106†
	kilogram-calories	kilogram-calories	kilogram-calories	kilogram-calories	kilogram-calories
- ΔH of carbohydrate fermented....	302.5	302.5	302.5	302.5	302.5
$\Sigma - \Delta H$ products \times mole fraction of each.....	323.4	323.0	314.4	322.6	320.6
- ΔH of fermentation per mole carbohydrate.....	30.9	20.5	11.9	20.1	18.1
- ΔF° of carbohydrate fermented	219.0	219.0	219.0	219.0	219.0
$\Sigma - \Delta F^\circ$ products \times mole fraction of each.....	265.2	265.4	238.2	264.8	255.9
- ΔF° of fermentation per mole carbohydrate.....	46.2	46.4	19.2	45.8	36.9

* 175 moles glycerol formed in this fermentation.

† This water reacts with the sugar to form the products. The maltose was calculated as glucose; the sucrose as 50 millimoles of glucose + 50 millimoles of fructose.

with resulting changes in the products, the energy liberated also shows variations.

It is apparent from the data just discussed that for different fermentations $-\Delta H$ per mole sugar fermented varies from about 5

kg-cal. or less, to as high as 50 kg-cal., while $-\Delta F^\circ$ ranges from 20 to 70 kg-cal. It would appear that either the needs of organisms for energy are much different or that an excess is produced in a large number of cases. No definite conclusion can be drawn in regard to this point since the crop of bacteria for the individual fermentations was not determined. Only by a comparison of the energy available per unit organism could the minimum require-

TABLE 13
*Energy balance of *Cl. acetobutylicum* on carbohydrates (31)*

CARBOHYDRATE	GLUCOSE	MANNITOL	ARABINOSE
	millimoles	millimoles	millimoles
Total fermented.....	1000	1000	1000
Products formed:			
Acetic acid.....	100	275	220
Butyric acid.....	12	153	3
Acetone.....	313	78	394
Ethyl alcohol.....	288	60	247
Butyl alcohol.....	489	604	293
Carbon dioxide.....	2282	2070	1702
Hydrogen.....	1382	2140	946
Water.....	122	263	216
— ΔH of substrate fermented.....	302.5	317.9	253.8
$\Sigma - \Delta H$ products \times mole fraction of each.....	314.5	322.8	265.5
— ΔH of fermentation per mole substrate	12.0	4.9	11.7
— ΔF° of substrate fermented.....	219.0	226.1	184.4
$\Sigma - \Delta F^\circ$ products \times mole fraction of each.....	277.8	282.5	231.7
— ΔF° of fermentation per mole substrate	58.8	56.4	47.3

ments for bacterial activity be ascertained. A second point noticed in these data is that $-\Delta F^\circ$ is two to three times as large as $-\Delta H$. This means that the organisms *may* have more energy available for work than is indicated by the heat of reaction. This additional energy would come from the environment. It must be emphasized that this does not mean that the organisms themselves possess the ability to convert heat into chemical energy, but only that the reactions taking place by reason of the activity

of the bacteria can proceed in a manner that will convert heat into free energy. Whether this actually occurs or not will depend upon the degree of reversibility of the system—the calculated values are the maximum free energy available and do not tell us how much of this is actually available. It is of interest to note that Burk (12) reports a similar finding for the lactic acid-glycogen system in the muscle.

III. THE ENERGETICS OF NITROGEN FIXATION BY BACTERIA

Nitrogen fixation by Azotobacter

The energy changes in the fixation of atmospheric nitrogen by bacteria have been the subject of much controversy. No doubt this is due to the lack of knowledge regarding the mechanism of the fixation as well as the contradictory nature of the data in regard to the carbon:nitrogen ratio. However, in the case of fixation of nitrogen by Azotobacter there is sufficient information available to hazard an estimate of the efficiency of this organism in its synthetic activity. Kostytschew (35) and his coworkers claim that Azotobacter fixes atmospheric nitrogen by reduction, i.e., ammonia is the first product formed, and offer some experimental evidence for this view. Linhart (41) calculated the efficiency of the organism to be about 1 per cent. He assumed that ammonium hydroxide was formed from nitrogen and water, but does not give the equation for its formation. The reaction is usually written,



a synthesis that is very unlikely since $-\Delta F^\circ = -78.5$ kg-cal. Calculations show that with an equilibrium concentration of 10^{-4} moles of NH_4^+ and OH^- and a partial pressure of 0.2 atm. of O_2 , a fugacity (pressure) of about 10^{45} atm. of nitrogen would be required. A second criticism is the fact that he used standard free energy values for all the reactions, a condition that obviously does not exist in the sphere of influence of the organism. Finally, the data used for the C:N ratio is misleading. While for practical purposes it may be considered that the energy used for nitrogen fixation is only about 1 per cent of that liberated in the com-

bustion of the carbohydrate, still this is not the problem with which we are concerned. There is no doubt but that a large amount of the energy that is set free by the organism in the burning of the carbon source is used for purposes other than nitrogen fixation. Energy for the latter must be differentiated from that used in cell maintenance, growth, work, etc. The work of Ome-liansky (52), Koch and Seydel (34) and others indicate that in young cultures the C:N ratio is greatly decreased. Koch and Seydel in one series of experiments secured an average of 75 mg. nitrogen fixed per gram of carbohydrate used. If we consider only the energy that is involved in the fixation of nitrogen, it follows that under optimum conditions, the efficiency of Azotobacter is probably much higher than 1 per cent and very likely compares favorably with that of the autotrophic organisms. The work of Meyerhof and Burk (46) confirms this. By an application of thermodynamics to the probable reactions that take place in the fixation of nitrogen by Azotobacter, it was shown that 4.81 moles nitrogen should be fixed per mole of sugar used and that $\frac{\text{cc. N}_2 \text{ fixed}}{\text{cc. O}_2 \text{ used}}$ should have a maximal value of 0.915.

Meyerhof and Burk studied the consumption of oxygen and the fixation of nitrogen by 24 to 48 hour cultures of Azotobacter over periods of 6 to 12 hours by the use of micro methods. With normal oxygen tension (0.21 atm.) the efficiency ratio (cc. N₂ fixed/cc. O₂ used) was found to be only 0.0025 to 0.008. However, with a change in the gas pressures, especially that of the oxygen, they found that this efficiency ratio could be increased to as high as 0.10. It appeared that the lower the oxygen tension, the higher is the efficiency, although the absolute amount of nitrogen fixed was markedly decreased if the oxygen tension was 0.01 atm. (5 per cent of normal) or lower. With an oxygen tension of 0.05 atm. (25 per cent of normal) the absolute fixation was increased two- to six-fold and an efficiency of 0.01 to 0.02 was obtained. These results indicate that under optimum conditions the "machine efficiency" of the organism with regard to nitrogen fixation varies from 2.5 to 10 per cent.

Burk (13, 14) has recently published results of further studies

on the relationship of the pressure of oxygen and nitrogen to the efficiency of nitrogen fixation by Azotobacter. He found that in an atmosphere containing 20 per cent oxygen, it was necessary to have at least 6 per cent nitrogen in order that fixation might take place. Up to 0.5 atm. (50 per cent) of nitrogen, the rate of growth (or fixation) was approximately directly proportional to the pressure of this gas. The maximum fixation occurred at 5 to 10 atm. of nitrogen. The presence of humic acid markedly increased the rate and efficiency of fixation and decreased the limiting nitrogen pressure to less than 1 per cent (0.01 atm.). If ammonia nitrogen were added in order to prevent the assimilation of elemental nitrogen, the stimulative action was still evident. Therefore, Burk concluded that the direct effect of humic acid was on the growth metabolism of the bacteria and not on the fixation. The efficiency of the fixation as measured by mg. nitrogen fixed/mg. glucose consumed was increased from 0.25 per cent to 0.72 per cent when the initial glucose concentration was decreased from 10 per cent to 1 per cent. Burk also used the ratios (1) $K = \text{count increase/total count} + \text{respiration rate increase/initial respiration rate}$, and (2) cell number increase/respiration due to growth, as measures of the efficiency of the organism. With these criteria, he found a marked increase in efficiency as the pressure of oxygen was lowered below 0.05 atm. However, this increase with the lower pressures was independent of nitrogen fixation. Under all conditions, the efficiency of growth (or efficiency of fixation) was decidedly increased with an increase in the rate of growth (or rate of fixation). As a result of these findings, Burk concludes that: (1) the oxygen pressure functions of Azotobacter, with respect to growth, growth efficiency, respiration and humic acid, have nothing to do with the actual process of fixation; (2) the nitrogen pressure affects solely the fixation and not the general growth metabolism of the organism.

Burk (11b) in a discussion of the energetics of nitrogen fixation by Azotobacter concludes from these studies on growth and fixation efficiency that probably the first product of fixation occurs in a reaction requiring little, if any, energy. He points out that

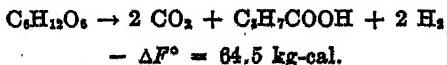
if Azotobacter is grown in free or fixed nitrogen under conditions in which the rates of growth are equal, equal quantities of energy (supplied by the oxidation of glucose) are required to produce equal dry weights of the organisms. This means that the efficiencies of growth in free and fixed nitrogen are equal, and therefore, the energy consumption required for the overall process of fixation from nitrogen gas to ammonia (as distinguished from simply the first step) is small, or zero as far as the experiments are capable of deciding. Moreover under optimum conditions for efficient growth and fixation (very low oxygen tension, etc.) 1 gram of sugar will suffice to fix 0.1 gram of nitrogen and produce 1 gram of dry weight of growth. Since most aerobic organisms require 2 to 10 grams of carbohydrate to produce 1 gram of dry weight, it would appear likely that this 1 gram of sugar used by Azotobacter is required solely for growth purposes and practically none is used in the fixation. All the experiments showed that when Azotobacter is grown in air, its respiration is such that much more energy is available than is actually required for growth and maintenance, but none of this excess energy can be charged to nitrogen fixation since the surplus occurs whether or not nitrogen is being fixed. Burk shows in addition that the first step in the fixation concerns a compound that is in equilibrium with the nitrogen of the air. This key compound is formed instantaneously from free nitrogen as rapidly as it is used in growth, as the concentration present at all times is approximately proportional to the pressure of nitrogen up to 0.5 atm. Hence its formation is represented by an equilibrium reaction with high reversibility, which means that the free energy involved in its formation is substantially zero. The formation of this compound (without expenditure of energy) is fixation in the strictest sense of the term. Subsequent conversions of this initial or key compound into the Azotobacter cell might involve free energy changes that are irreversible and hence require some energy, but this possibility is presumably ruled out by the data presented earlier in the paragraph.

From a consideration of the foregoing discussed papers, it appears that the value assigned for the efficiency of Azotobacter

in the fixation of free nitrogen depends to a great extent upon experimental conditions and, of course, upon the definition of efficiency employed. In view of this variability, it appears to be the better part of valor to avoid a definite statement as to the efficiency of nitrogen fixation in nature by this organism. On the other hand, the progress made toward a solution of this problem by Meyerhof, Burk and their collaborators, makes it appear hopeful that the answer will be available in the near future.

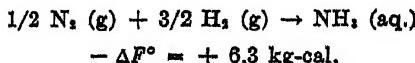
*Nitrogen fixation by *Cl. pasteurianum**

Little work has been done on the energetics of the fixation of nitrogen by the anaerobic species *Cl. pasteurianum*. This fixation is usually considered as one of very low efficiency, since the average value of nitrogen fixed per gram of carbohydrate used is 1 to 2 mg. However, aside from the question of what proportion of this energy is used in the actual fixation, the fact that the carbohydrate is only partially degraded must not be forgotten. For example, if glucose is broken down by this organism according to the equation,



$-\Delta F^\circ$ is less than one-tenth the corresponding value for the complete combustion of glucose to carbon dioxide and water brought about by the aerobic species Azotobacter (+685.8 kg-cal.). Neglecting, in the above reaction, the changes in $-\Delta F$ incident to change of concentration of the resultants and reactant, it may be readily seen that *Cl. pasteurianum* is as efficient, if not more so, in its utilization of available energy than is Azotobacter. The apparent inefficiency is due to the comparison of the nitrogen fixed to the carbohydrate that disappears, which ignores the butyric acid with its high energy content formed in the metabolism of this anaerobic organism. However it is by no means certain that the aerobic and anaerobic nitrogen fixers employ the same mechanism for the fixation. Because of the large quantities of hydrogen that are liberated in the destruction

of carbohydrate by anaerobic organisms of the Clostridium type, it seems probable that the fixation would take place thus:



If this reaction occurs in the cell, the value of $-\Delta F$ would be changed according to the activities (concentrations) of the various substances concerned. On the basis of this equation Buchanan and Fulmer (10) have calculated the molality of the ammonia in equilibrium with 0.8 atm. of nitrogen and varying partial pressures of hydrogen. At a pressure of 10^{-4} atm. hydrogen (the partial pressure of hydrogen in the air according to Burk (11)) the equilibrium concentration of ammonia would be $3.69 \times 10^{-2} M$. Since the reaction takes place in the liquid phase, the fixed nitrogen would appear as ionized NH_4OH . In this case the pH of the cell would affect the equilibrium concentration of the NH_4^+ . At neutrality, with a pressure of 0.8 atm. of nitrogen and 10^{-4} atm. of hydrogen, the NH_4^+ could reach a concentration of $11.9 \times 10^{-1} M$ before the sign of the free energy change becomes positive. The equilibrium concentration increases tenfold for each unit change in pH toward the acid side and decreases tenfold for each unit change in pH toward the alkaline side. From these data it appears possible that the fixation of nitrogen by the anaerobic species may be exothermic. The energy derived from the utilization of carbohydrate is used for purposes other than nitrogen fixation.

Nitrogen fixation by Rhizobium

The study of the energetics of nitrogen fixation is even more restricted by lack of suitable and reliable data in the case of the symbiotic forms of bacteria, than it is in that of the free-living forms—Azotobacter and Clostridium. Since it has not been conclusively demonstrated that the nodule bacteria can fix nitrogen independent of the plant, the employment of experimental data gained in the few cases in which nitrogen was reported fixed in pure culture would be of doubtful value. In addition, the mechanism of fixation is by no means established. Studies of

nitrogen fixation in connection with the plant are complicated by the carbon and nitrogen metabolism of the latter, so that suitable technique has not been developed that would make possible a differentiation of energy used by the organism and that by the plant. If we reason by analogy that the fixation is similar to that of Azotobacter and the efficiency is of the same order, the conclusions reached are highly improbable. Thus Christiansen-Weniger (15) has shown that, for typical field experiments, the energy utilized for fixation would require a carbohydrate destruction of two to three times the total yield of the plants unless the process of fixation is much more efficient than is that of Azotobacter. He believes that the fixation must occur by an exother-

TABLE 14
Energy requirements for the fixation of nitrogen by legumes
 Christiansen-Weniger

PLANT	TOTAL DRY WEIGHT		DIFFER- ENCE	NITRO- GEN FIXED	ENERGY REQUIRED FOR FIXA- TION OF 1 KILOGRAM NITROGEN
	Without nitrogen	With nitrogen			
	grams	grams	grams	grams	kilogram- calories
Bean.....	50.746	56.572	5.826	1.021	22,800
Alfalfa.....	60.659	68.487	7.828	1.184	28,884

mic process and offers experimental evidence in support of this. He found that the total dry weights of lupines, beans, and alfalfa grown in the presence of sodium nitrate showed only slight increases over inoculated plants grown in the absence of fixed nitrogen. From the small differences obtained, he calculated the energy utilized for fixation of the nitrogen on the basis of the equivalence of 1 gram dry plant material to 4.0 kg-cal. A typical set of results is given in table 14.

On the basis of these results, Christiansen-Weniger concludes that the amount of energy required in the fixation is so small that it is probable that this energy is used for the vital activity of the bacteria and that the actual fixation of nitrogen is accomplished by an exothermic reaction that yields energy to the

bacteria for their cell processes. However, the energy required for the fixation is not particularly small, as can be seen from the above table.

Rippel and Poschenrieder (56) take issue with Christiansen-Weniger in regard to this proposed autotrophic nature of the nodule bacteria. They observed an accumulation of starch in nodules while the plant was engaged in carbon assimilation which disappeared if the latter process was interfered with, as by transferring the plants to the dark. They regarded this accumulation of starch in the nodules as a means of supplying the needs of the bacteroids in fixing the nitrogen. They pointed out two sources of error in Christiansen-Weniger's work: (1) The nitrogen in the control was supplied as nitrate, which would require energy for reduction; (2) the difference in dry weight at the end of the experiment between the controls and plants that were inoculated is not necessarily a measure of the total carbon that was used in the fixation. It has been observed that the assimilation ability of the legumes for a given leaf area is twice that of other plants, and that if some of the leaves are prevented from assimilating carbon dioxide there is a compensatory increase on the part of the other leaves. Hence Rippel and Poschenrieder claim that the nodules could use a great deal more carbohydrate than was shown by Christiansen-Weniger's results and still have little influence on the final dry weight of the plant. Added evidence that carbohydrate is consumed in the nodules is furnished by the observation of Reinau that the "earth respiration" of legumes is much higher than that of mustard or of rye.

Rippel and Poschenrieder believe the hydrogen for the reduction of the nitrogen arises from a fermentation process similar to that supplying the hydrogen used in the production of alcohol by yeast. In this case, for every 180 grams of glucose used, 4 grams of hydrogen (fermentation) are produced. If this hydrogen were used entirely for reduction of nitrogen to ammonia, then a fixation of about 100 mg. per gram of sugar destroyed would result, which is of the same order as that fixed by young cultures of Azotobacter.

Burk (11) has made a theoretical study of the various mecha-

nisms suggested for the fixation of nitrogen by *Rhizobium* in the nodule of legumes and by the free-living forms, e.g., *Azotobacter* and *Cl. pasteurianum*. He stresses the point of view that the actual fixation in the primary compound is the question involved, and that subsequent reactions in which this first compound is concerned are a part of the nitrogen metabolism and for this reason the energy changes involved should be discriminated from those of fixation. By an application of thermodynamics to the various mechanisms proposed for the fixation, he shows that the fixation might take place by the reduction of nitrogen to ammonia, by oxidation to nitric acid, or by the formation of simple organic compounds and under certain conditions, all these mechanisms could yield energy and thus free the organism from the necessity of obtaining energy by the oxidation of carbohydrate. The sign of $-\Delta F$ of most of the proposed reactions for the mechanism is positive, provided the calculations are based on concentrations likely to be present in the cell rather than on the standard free energy equation. Since a number of these reactions require hydrogen, Burk suggests that this gas might be furnished by the small quantity in the atmosphere, 0.01 per cent, or by that arising from fermentation of carbohydrate by the cell. In the latter case, the energy loss suffered due to the hydrogen being liberated and not oxidized cannot be charged to nitrogen fixation since this liberation takes place irrespective of the fixation.

SUMMARY

The energy liberated in the metabolic processes of bacteria may be from two to one hundred times as much as is represented by the cells. Part of this is immediately converted into heat because of the irreversibility of the processes carried out by the organisms. A portion of the free energy available for work is used for cell growth and synthesis. Motion apparently requires only a small part of the total available energy. It appears that most of the free energy: (1) is transformed into surface energy; (2) performs osmotic work; (3) displaces equilibrium conditions within the cell; (4) possibly maintains oxidation-reduction potentials. Eventually the energy appears as heat.

The heat of reaction ($-\Delta H$) of fermentations carried out by different species of bacteria varies from 5 kg-cal. or less, to over 50 kg-cal. per mole of carbohydrate fermented. The standard free energy ($-\Delta F^\circ$) of a number of reactions studied is two to three times greater than the heat of reaction.

The energetics of nitrogen fixation by the aerobic species, Azotobacter, indicate that an efficiency of at least 10 per cent is reached under optimum conditions of fixation. The anaerobic species, *Cl. pasteurianum*, is equally efficient if energy liberated rather than carbohydrate used is taken as a criterion. It is probable that the actual fixation takes place exothermically by reduction of nitrogen under anaerobic conditions.

The data on the fixation of nitrogen by the legume bacteria in association with the plant suggest that the process is probably more efficient than with Azotobacter, and it is possible that the actual fixation occurs by an exothermic reaction.

In conclusion it is apparent that the field of bacterial energetics suffers from a lack of precise and adequate data. The results of Rubner and Tangl in the investigation of the general problem need to be checked by studies in which more modern technique is employed. The application of thermochemistry, calorimetry, and thermodynamics to a number of specific fermentations should yield valuable information for an understanding of these processes.

The authors wish to thank Prof. Warren Weaver, Prof. F. Daniels, Dr. Dean Burk, and Dr. K. K. Kelley for their aid and suggestions in various parts of this paper.

REFERENCES

- (1) ANGERER, V.: Arch. Hyg. 88, 139 (1919).
- (2) BAAS-BECKING, M. AND PARKS, G. S.: Physiol. Rev. 7, 85 (1927).
- (3) BAYNE-JONES, S.: J. Bact. 17, 105 (1929).
- (4) BAYNE-JONES, S. AND RHEES, H. S.: J. Bact. 17, 123 (1929).
- (5) BARON, J. AND POLÁNYI, M.: Biochem. Z. 53, 1 (1918).
- (6) BEEBE, S. P. AND BUXTON, B. H.: Am. J. Physiol. 12, 466 (1905).
- (7) BRAAK, H. R.: Onderzoeken over vergisting van Glycerine. Doctoral Thesis, Delft, 1928.
- (8) BROWN, H. T.: Ann. Botany 28, 197 (1914).
- (9) BUCHANAN, R. E. AND FULMER, E. I.: Physiology and Biochemistry of Bacteria, vol. I. Williams and Wilkins Company, Baltimore (1928).

- (10) BUCHANAN, R. E. AND FULMER, E. I.: *Physiology and Biochemistry of Bacteria*, vol. III. Williams and Wilkins Company, Baltimore (1930).
- (11) BURK, D.: *J. Gen. Physiol.* 10, 559 (1927).
- (11a) BURK, D.: *J. Phys. Chem.* 35, 432 (1931).
- (11b) BURK, D.: *Proc. Intern. Soil. Sci. Congress*, Leningrad, 1930. In Press.
- (12) BURK, D.: *Proc. Roy. Soc. (London)* 104B, 153 (1929).
- (13) BURK, D.: *J. Phys. Chem.* 34, 1174 (1930).
- (14) BURK, D. AND LINEWEAVER, H.: *J. Bact.* 19, 389 (1920).
- (15) CHRISTIANSEN-WENIGER, F.: *Centr. Bakt. Parasitenk., Abt. II*, 58, 41 (1923).
- (16) DAVIS, N.: *J. Bact.* 13, 381 (1927).
- (17) DONNAN, F. G.: *J. Gen. Physiol.* 8, 685 (1926).
- (18) FOOTE, M., PETERSON, W. H. AND FRED, E. B.: *Centr. Bakt. Parasitenk., Abt. II*, 82, 379 (1930).
- (19) FULMER, E. I. AND LEIFSON, E.: *Iowa State J. Sci.* 2, 159 (1928).
- (20) GAYON, U. AND DUBOURG, E.: *Ann. inst. Pasteur* 15, 527 (1901).
- (21) GIAJA, J.: *Compt. rend. soc. biol.* 83, 1479 (1920).
- (22) GREY, E. C.: *Proc. Roy. Soc. (London)* 87B, 472 (1914).
- (23) GUYE, C. E.: *Physico-chemical Evolution*. Translated by J. R. Clarke. E. P. Dutton and Company, London (1925).
- (24) HARDEN, A. AND NORRIS, D.: *Proc. Roy. Soc. (London)* 85B, 73 (1912).
- (25) HARRINS, W. D.: In *Colloidal Behavior* (by Bogue, R. H.) vol. I, pp. 142-211. McGraw-Hill Book Company, New York (1924).
- (26) HERZOG, R. O.: *Z. physiol. Chem.* 37, 383 (1903).
- (27) HILL, A. V.: *J. Physiol.* 43, 260 (1911).
- (28) HILL, A. V.: *J. Physiol.* 44, 507 (1912).
- (29) HÖBER, R.: *Physikalische Chemie der Zelle und der Gewebe*, 5th edition. Wilhelm Engelmann, Leipzig (1924).
- (30) INGERSOLL, L. R. AND ZOBEL, O. J.: *Mathematical Theory of Heat Conduction*. Ginn and Company, Boston (1913).
- (31) JOHNSON, M. J., PETERSON, W. H. AND FRED, E. B.: *J. Biol. Chem.* 91, 569, June (1931).
- (32) KHARASCH, M. S.: *Bur. Standards J. Research* 2, 359 (1929).
- (33) KHARASCH, M. S. AND SHEB, B.: *J. Phys. Chem.* 29, 625 (1925).
- (34) KOCH, A. AND SEYDEL, S.: *Centr. Bakt. Parasitenk., Abt. II*, 31, 570 (1911).
- (35) KOSTYTSCHEW, S., RYSKALTSCHUK, A. AND SCHWEZOWA, O.: *Z. physiol. Chem.* 154, 1 (1926).
- (36) KRUSE, W.: *Allgemeine Microbiologie*. F. C. W. Vogel, Leipzig (1910).
- (37) LAME, H.: *Hydrodynamics*, 5th edition, p. 571. The Macmillan Company, Cambridge (1924).
- (38) LEWIS, G. N. AND RANDALL, M.: *Thermodynamics*. McGraw-Hill Book Company, New York (1923).
- (39) LIEBEN, F.: *Biochem. Z.* 135, 240 (1923).
- (40) LIEBEN, F. AND FÜRTH, O.: *Biochem. Z.* 132, 165 (1922).
- (41) LINHART, G.: *J. Gen. Physiol.* 2, 247 (1920).
- (42) LUNDIN, H.: *Biochem. Z.* 141, 310 (1923).
- (43) MEYERHOF, O.: *Chemical Dynamics of Life Phenomena*. J. B. Lippincott Company, Philadelphia (1924).
- (44) MEYERHOF, O.: *Pflügers Arch.* 146, 159 (1912).

- (45) MEYERHOF, O.: Biochem. Z. 35, 246 (1911).
- (46) MEYERHOF, O. AND BURK, D.: Z. physik. Chem. 139, 117 (1928).
- (47) MEYERHOF, O.: Biochem. Z. 162, 43 (1925).
- (48) MEYERHOF, O.: In Handbuch der Physik, Band XI, 8, 238. Julius Springer, Berlin (1926).
- (49) MUDD, S. AND MUDD, E. B.: J. Exptl. Med. 40, 647 (1924).
- (50) NATHANSON, A.: Kolloidchem. Beihefte 11, 261 (1919).
- (51) NERNST, W.: Theoretical Chemistry. The Macmillan Company, London (1923).
- (52) OMELIANSKY, V. L.: Arch. soc. biol. 18, 1 (1915).
- (53) OPPENHEIMER, C.: Handbuch der Biochemie, 2nd edition, vol. II. Gustav Fischer, Jena (1925).
- (54) PARKS, G. S. AND KELLEY, K. K.: J. Am. Chem. Soc. 47, 2089 (1925).
- (55) PARKS, G. S., KELLEY, K. K. AND HUFFMAN, H. M.: J. Am. Chem. Soc. 51, 1969 (1929).
- (56) RIPPEL, A. AND POSCHENRIEDER, H.: J. Landw. 76, 101 (1928).
- (57) RUBNER, M.: Arch. Hyg. 48, 280 (1903).
- (58) RUBNER, M.: Arch. Hyg. 49, 355 (1904).
- (59) RUBNER, M.: Arch. Hyg. 57, 193 (1906).
- (60) RUBNER, M.: Arch. Hyg. 57, 244 (1906).
- (61) RUBNER, M.: Arch. Physiol., Supplement, pp. 1-392 (1912).
- (62) SEBARGER, C.: J. Physiol. 55, 50 (1921).
- (63) STEPHENSON, M.: Bacterial Metabolism. Longmans, Green and Company, London (1930).
- (64) STYLES, H. R., PETERSON, W. H. AND FRED, E. B.: J. Biol. Chem. 44, 643 (1925).
- (65) TANGL, F.: Pflügers Arch. 98, 475 (1903).
- (66) TANGL, F.: Pflügers Arch. 115, 1 (1906).
- (67) VAN NEIL, C. B.: The Propionic Acid Bacteria. Doctoral Thesis, Delft, 1928.
- (68) VAN STEENBERGE, P.: Ann. inst. Pasteur 34, 803 (1920).
- (69) WACHTEL, C.: Pflügers Arch. 171, 66 (1918).
- (70) WAKSMAN, S. A.: Principles of Soil Microbiology. Williams and Wilkins Company, Baltimore (1927).
- (71) WARBURG, O.: Ergebnisse Physiol. 14, 253 (1914).
- (72) WETZEL, N. C.: J. Bact. 18, 117 (1929).
- (73) WOHLFEIL, T.: Centr. Bakt. Parasitenk., Abt. I, Orig. 117, 212 (1930).

